Figure 1

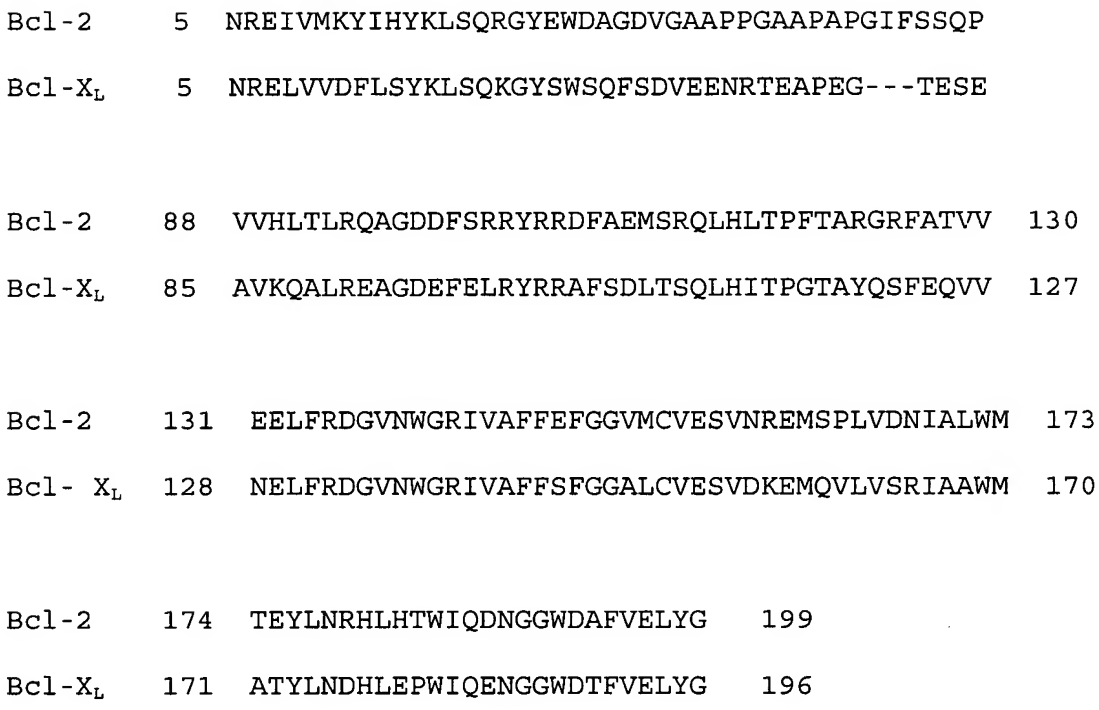

Bcl-2 5 NREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQP
Bcl-X_L 5 NRELVVDFLSYKLSQKGYSWSQFSDVEENRTEAPEG---TESE
Bcl-2 88 VVHLTLRQAGDDFSRRYRRDFAEMSRQLHLTPFTARGRFATVV 130
Bcl-X_L 85 AVKQALREAGDEFELRYRRAFSDLTSQLHITPGTAYQSFEQVV 127
Bcl-2 131 EELFRDGVNWGRIVAFFEFGGVMCVESVNREMSPLVDNIALWM 173
Bcl- X_L 128 NELFRDGVNWGRIVAFFSFGGALCVESVDKEMQVLVSRIAAWM 170
Bcl-2 174 TEYLNRHLHTWIQDNGGWDAFVELYG 199
Bcl-X_L 171 ATYLNDHLEPWIQENGGWDTFVELYG 196

Figure 2A

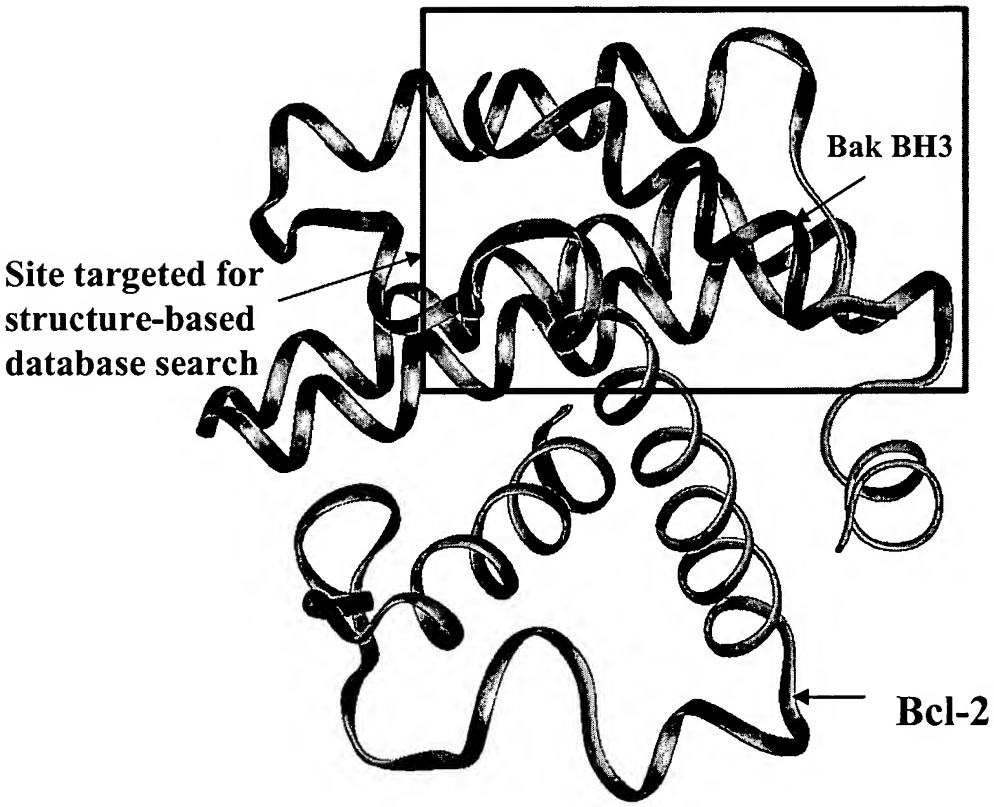

Bak BH3
Site targeted for
structure-based
database search
Bcl-2

Figure 2B

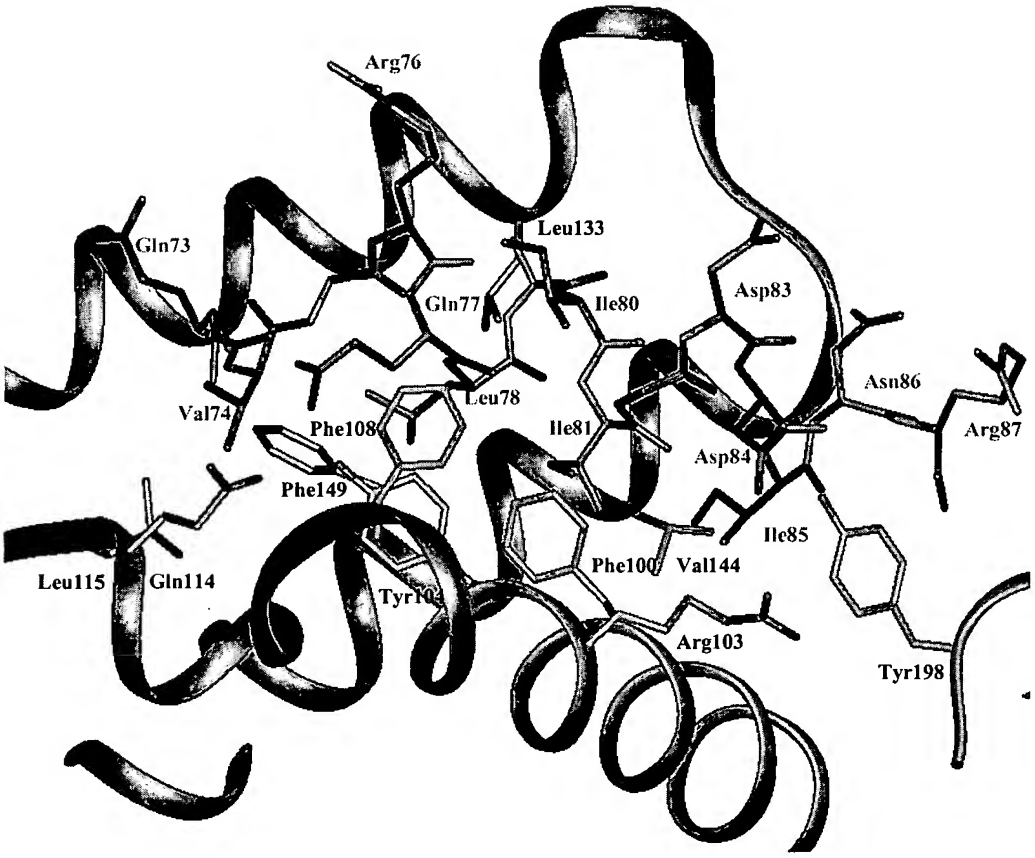

Arg76
Gln73
Leu133
Gln77
Ile80
Asp83
Asn86
Leu78
Val74
Phe108
Ile81
Arg87
Asp84
Phe149
Ile85
Phe100
Val144
Leu115
Gln114
Tyr104
Arg103
Tyr198

Figure 3

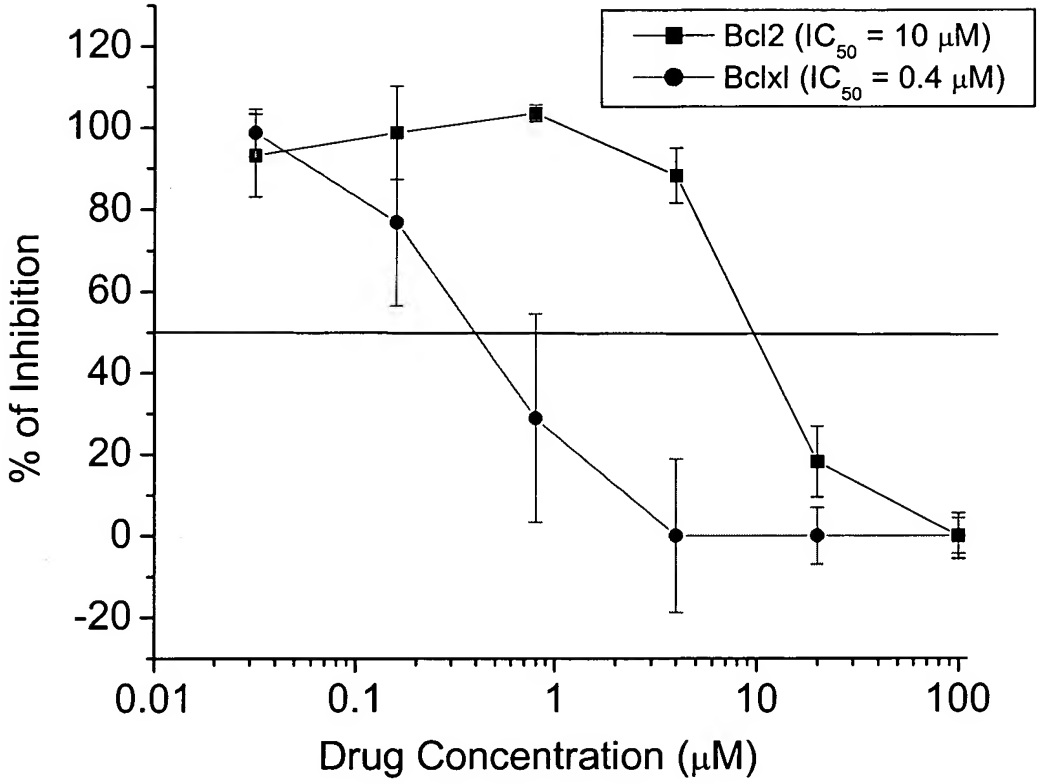

Bcl2 (IC50 = 10 μM)
Bclxl (IC50 = 0.4 μM)
% of Inhibition
120
100
80
60
40
20
0
-20
0.01
0.1
1
10
100
Drug Concentration (μM)

Figure 4

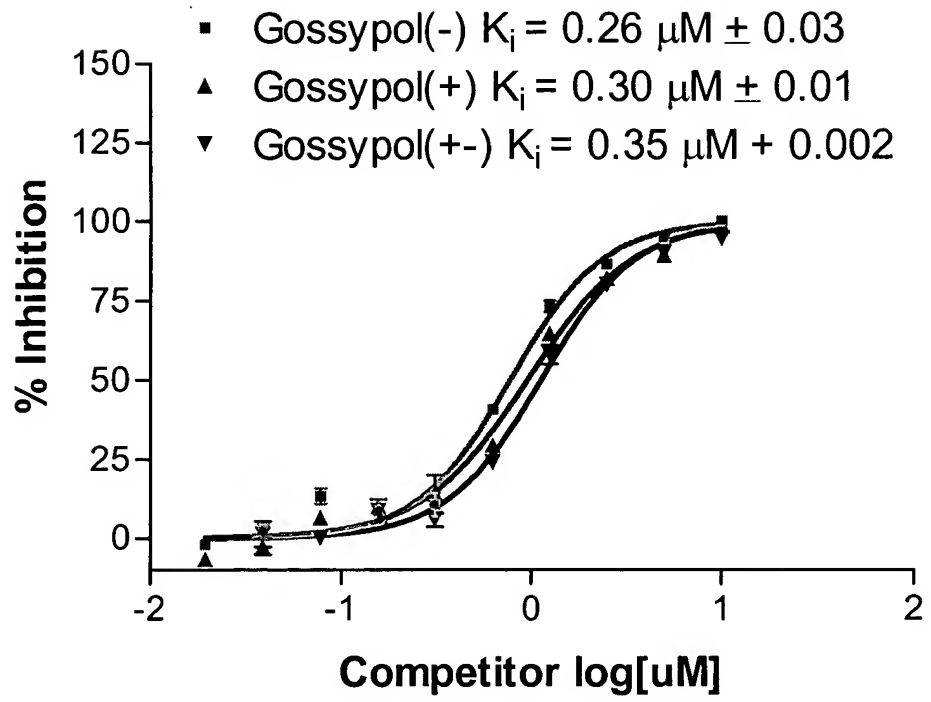

Gossypol(-) K_i = 0.26 μM ± 0.03
Gossypol(+) K_i = 0.30 μM ± 0.01
Gossypol(+-) K_i = 0.35 μM + 0.002
% Inhibition
150
125
100
75
50
25
0
-2
-1
0
1
2
Competitor log[uM]

Figure 5

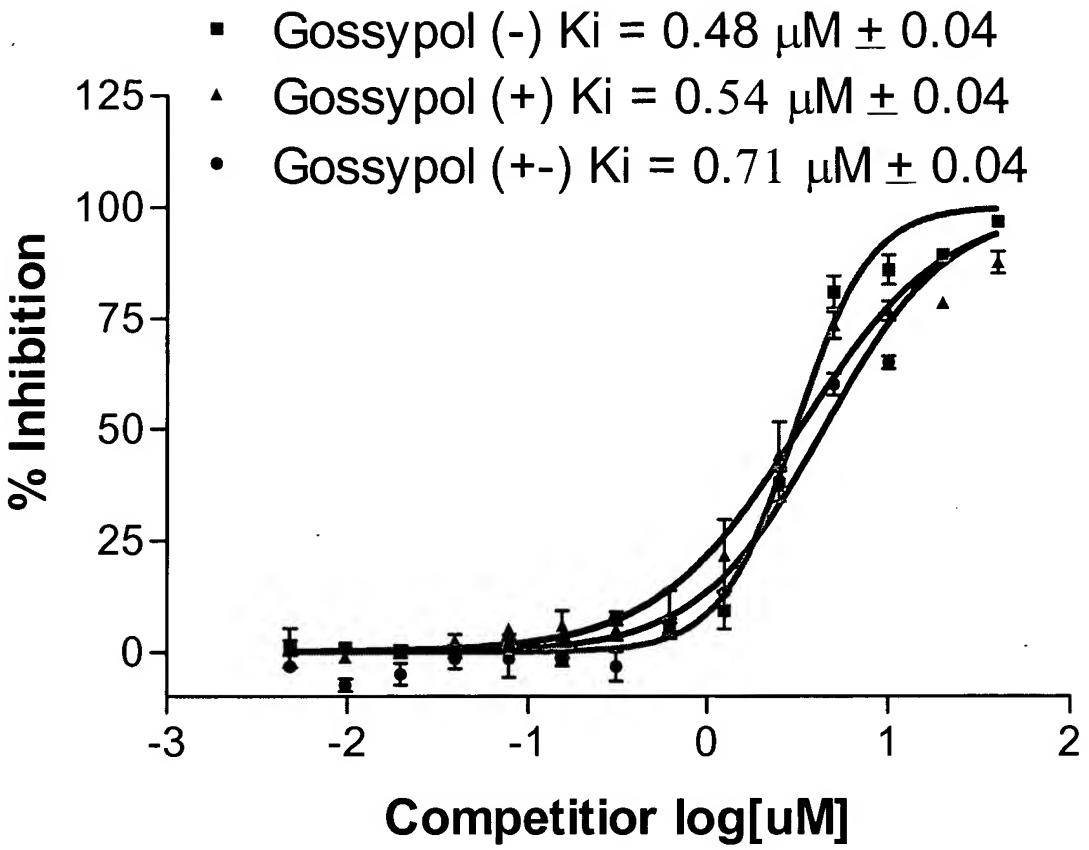

Gossypol (-) Ki = 0.48 μM ± 0.04
Gossypol (+) Ki = 0.54 μM ± 0.04
Gossypol (+-) Ki = 0.71 μM ± 0.04
% Inhibition
125
100
75
50
25
0
-3
-2
-1
0
1
2
Competitior log[uM]

Figure 6A

Binding of gossypolone to Bcl-X_L

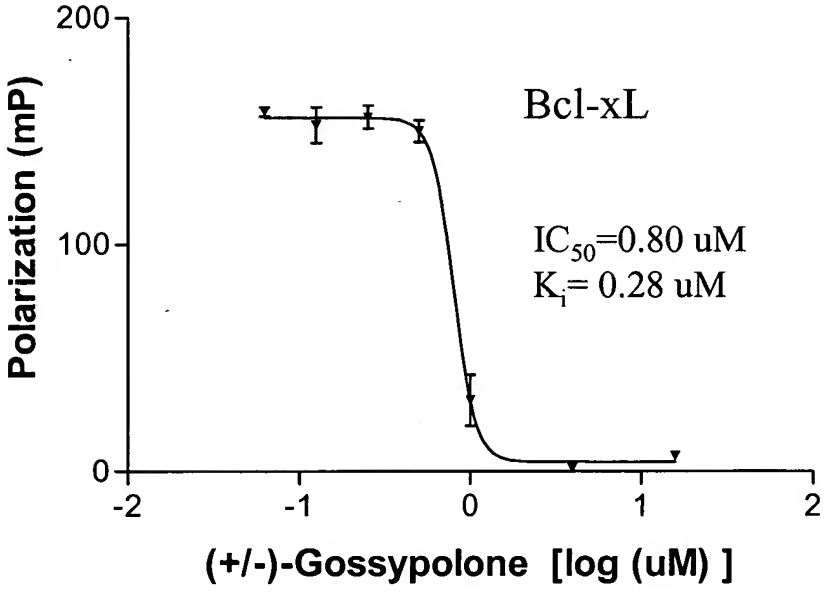


Figure 6B

Binding of Ethyl Schiff's base of (-)-Gossypol

IC_{50}	(after 18h30min)	7.346 uM
Ki		2.561 uM

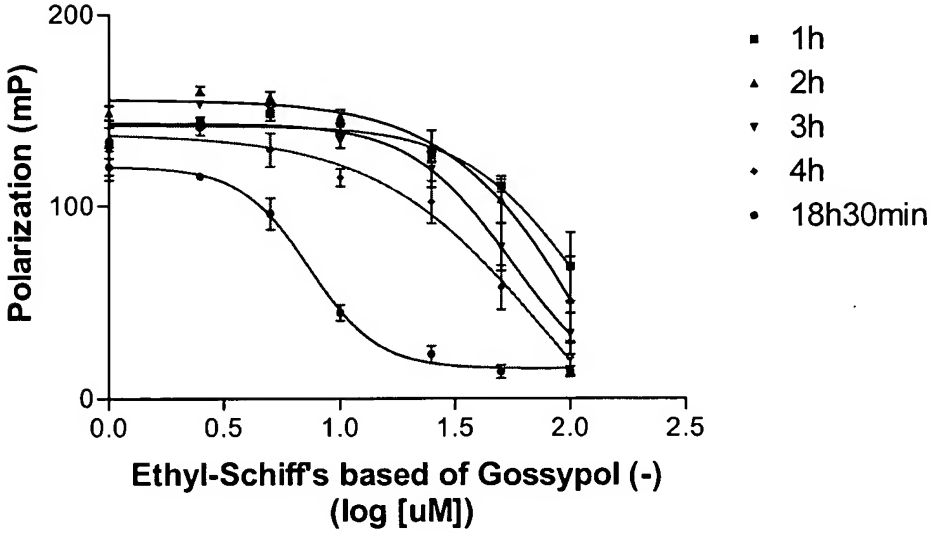
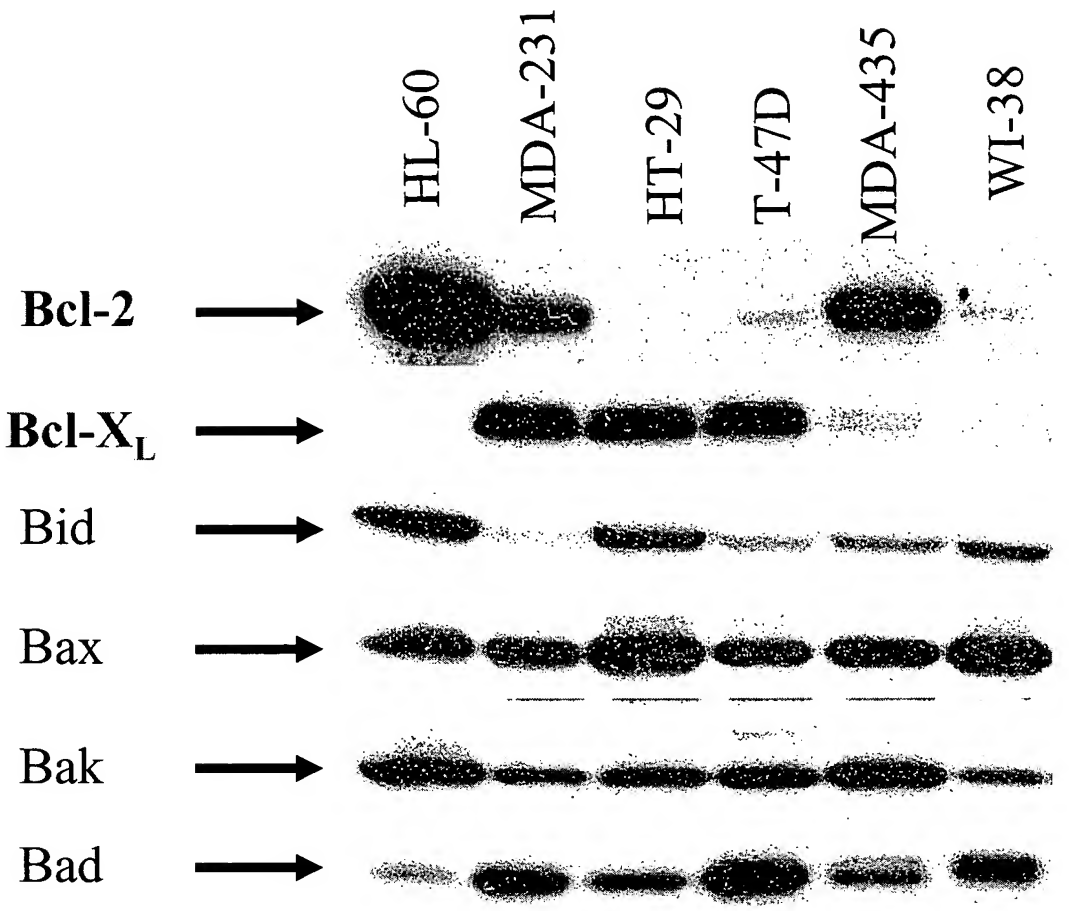
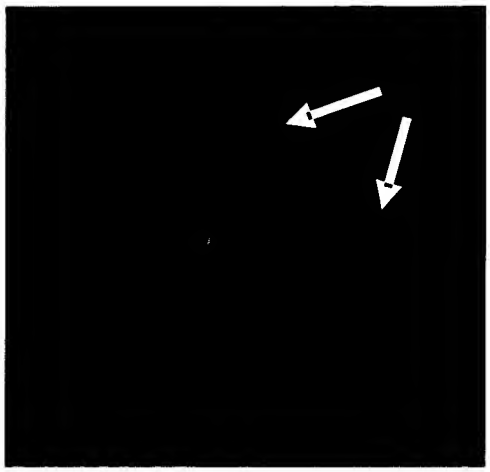


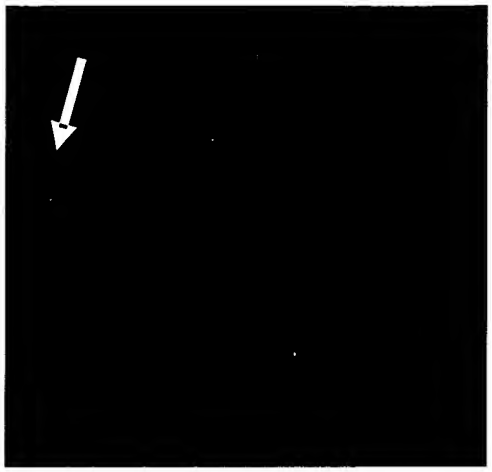
Figure 7





MDA-MB-231

Figure 8A



WI-38

Figure 8B

Figure 9

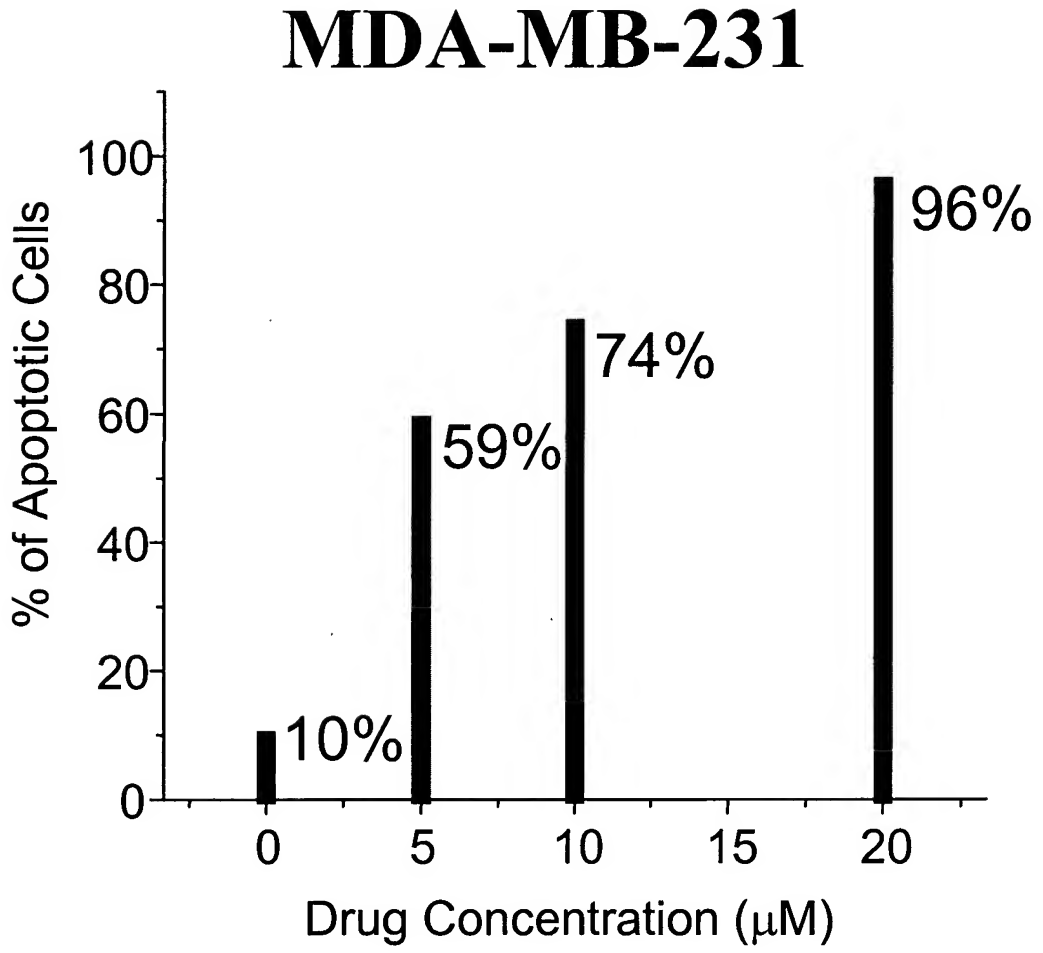

MDA-MB-231
% of Apoptotic Cells
100
80
60
40
20
0
10%
59%
74%
96%
0
5
10
15
20
Drug Concentration (μM)

Figure 10

T-47D

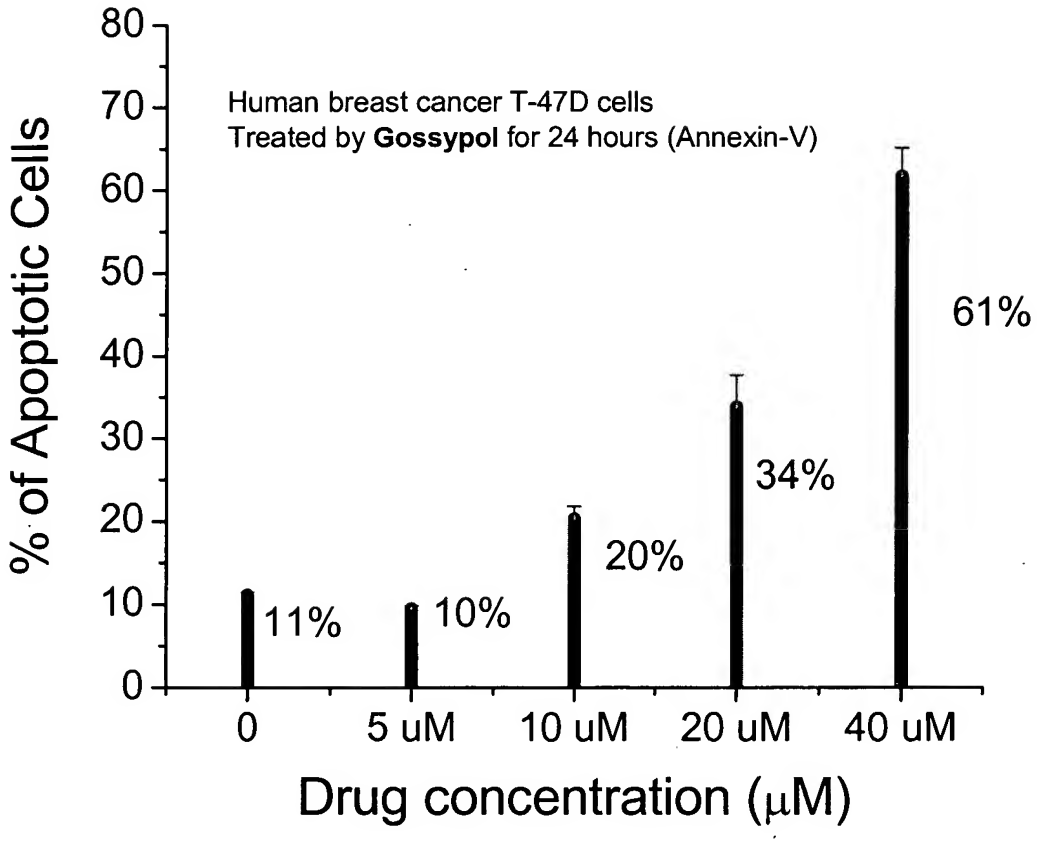


Figure 11A

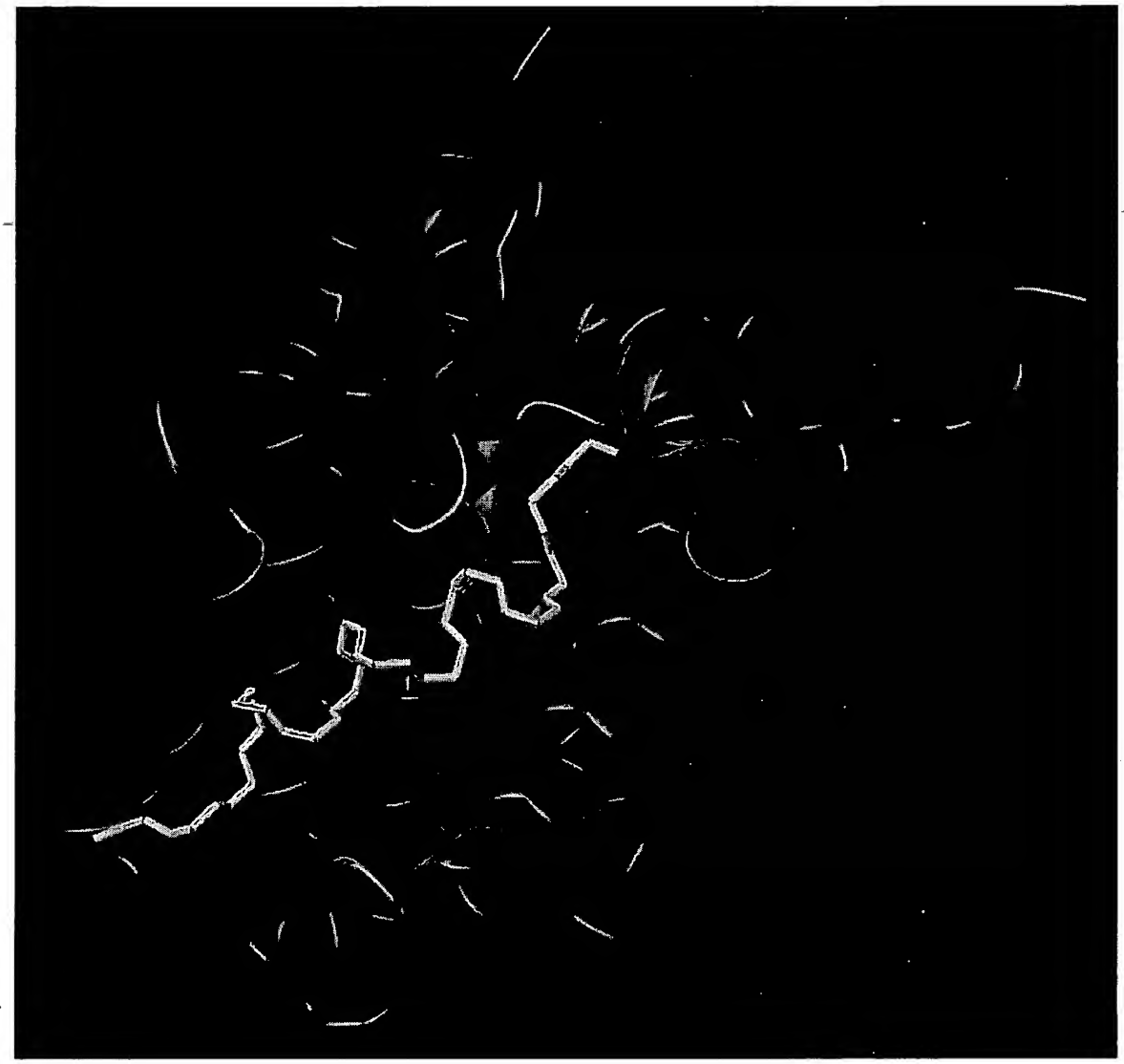


Figure 11B

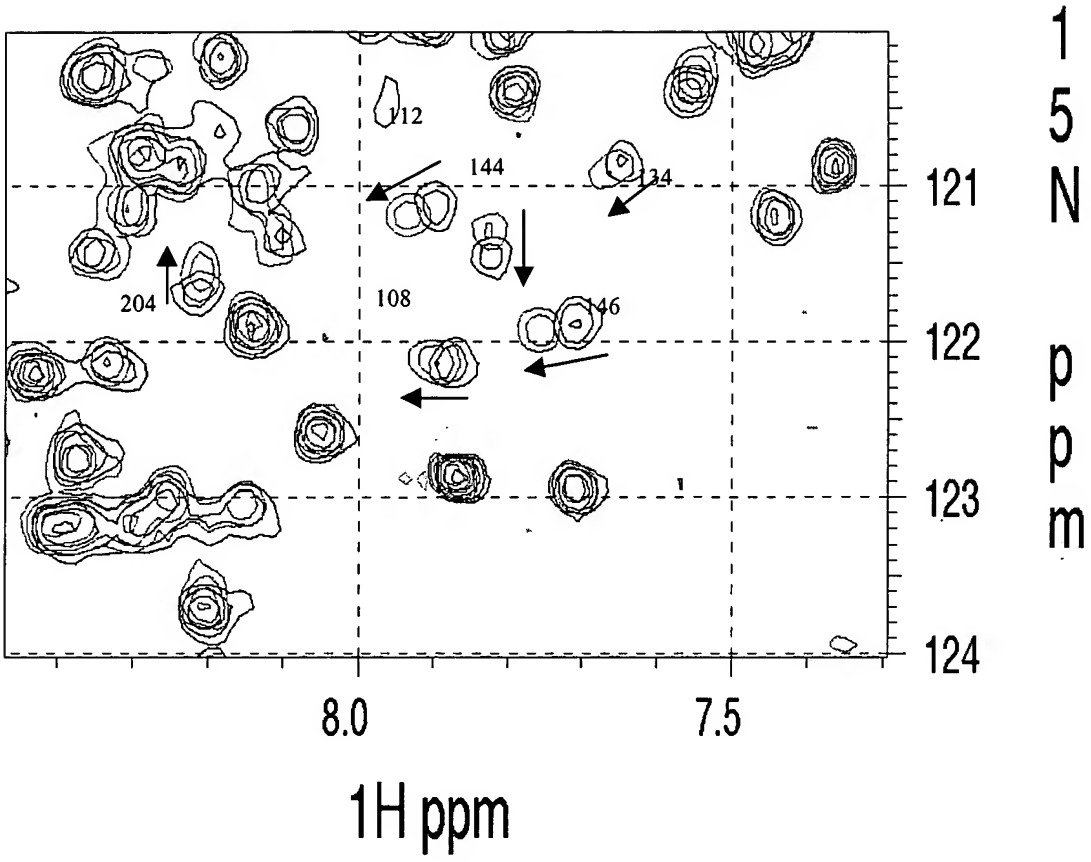


Figure 11C

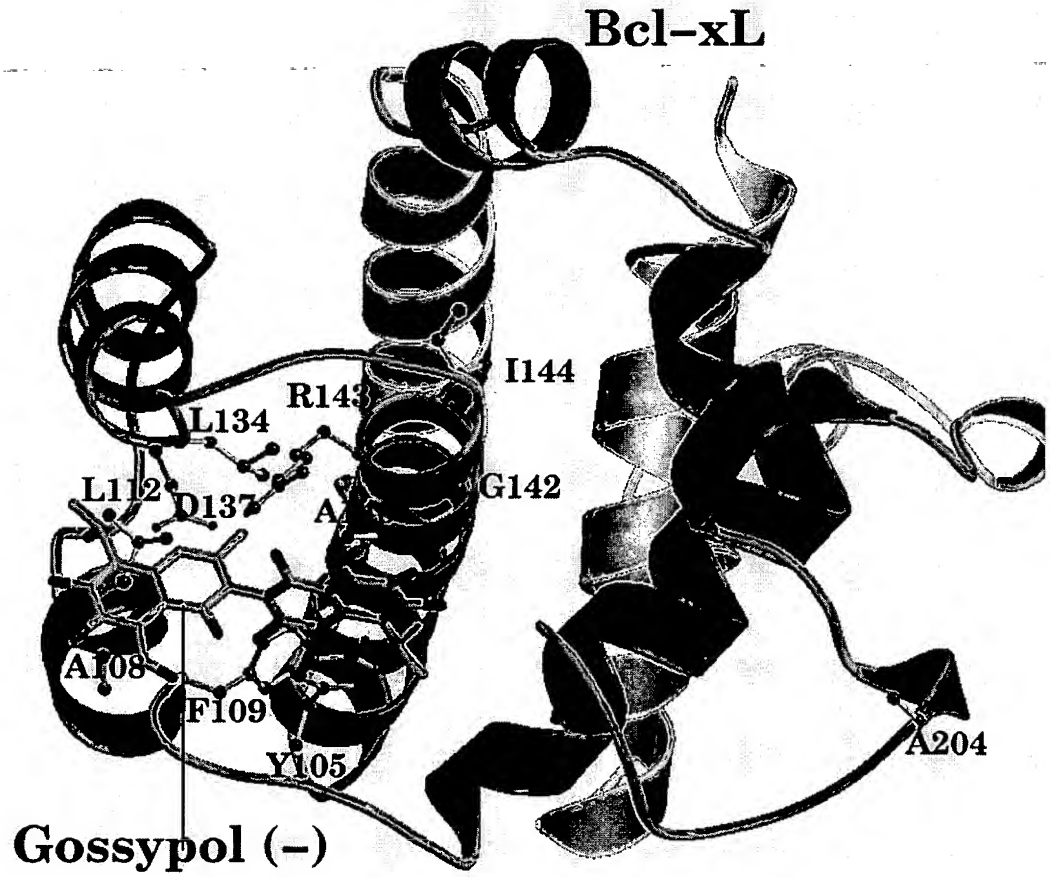

Bcl-xL
I144
R143
L134
G142
L112
D137
A108
F109
Y105
A204
Gossypol (-)

Figure 12

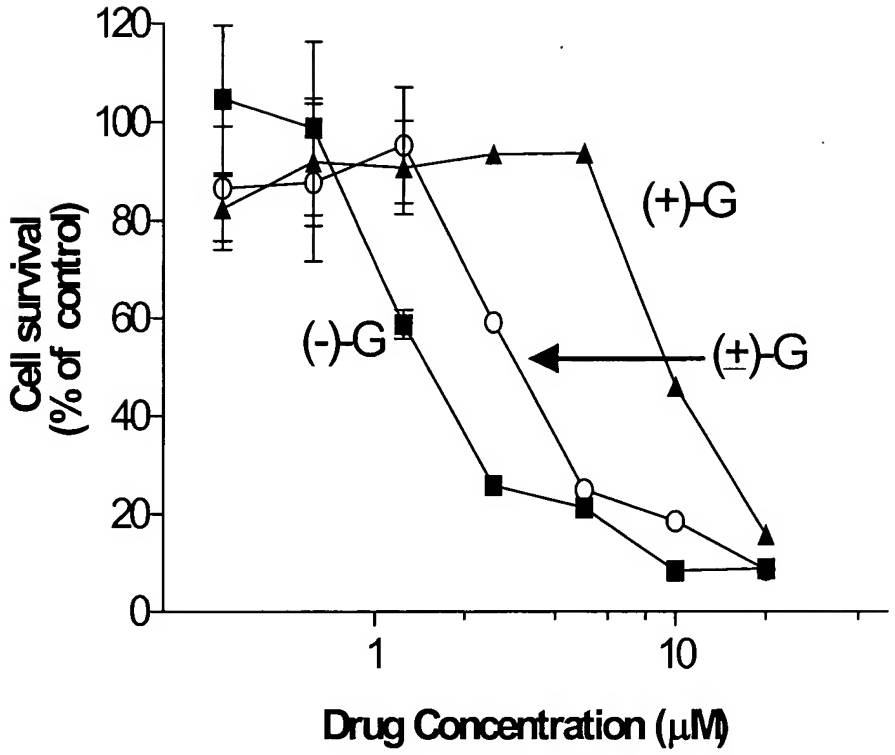

120
100
80
60
40
20
0
Cell survival
(% of control)
1
10
Drug Concentration (μM)
(+)-G
(-)-G
(±)-G

Figure 13

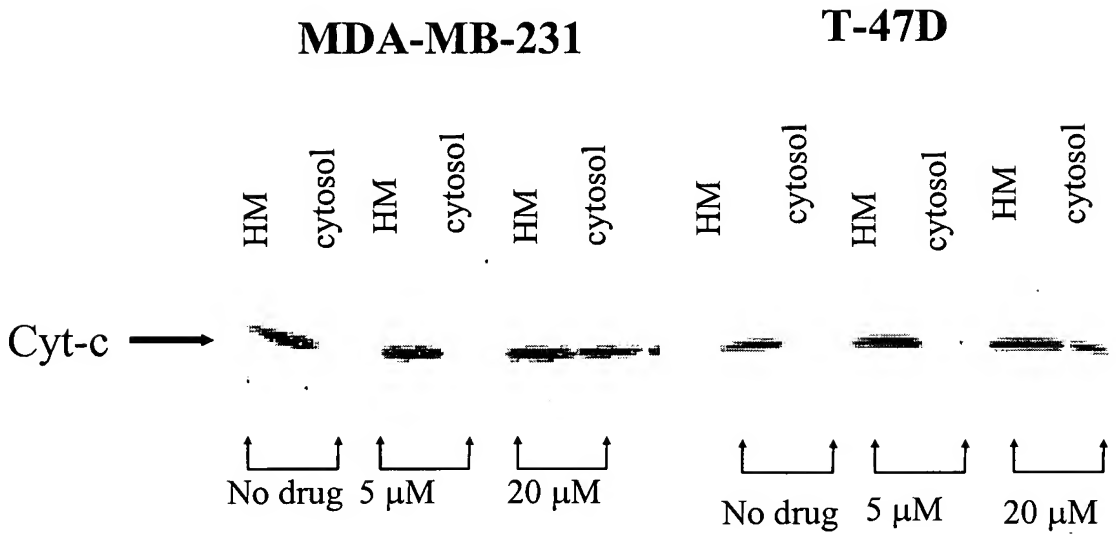

MDA-MB-231
T-47D
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
HM
cytosol
Cyt-c
No drug
5 μM
20 μM
No drug
5 μM
20 μM

Figure 14

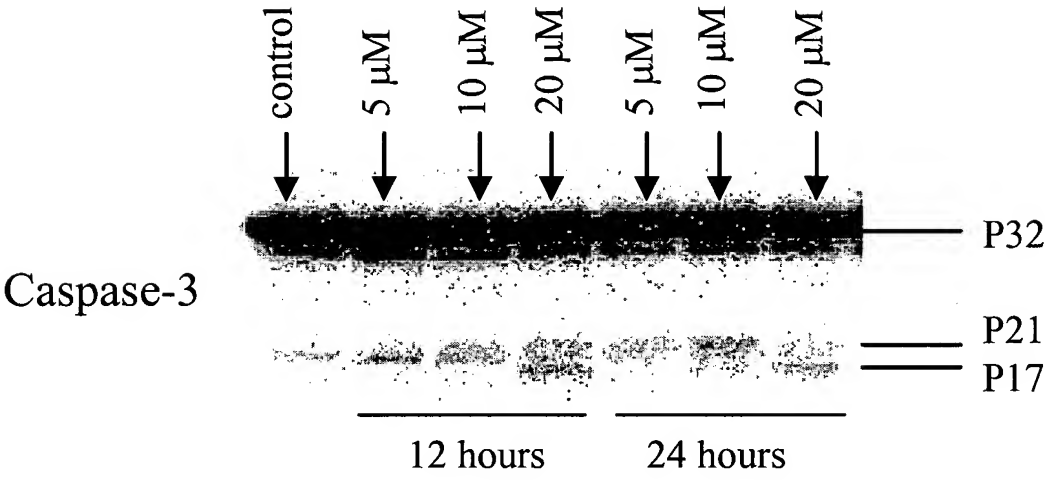

control
5 μM
10 μM
20 μM
5 μM
10 μM
20 μM
Caspase-3
P32
P21
P17
12 hours
24 hours

Figure 15

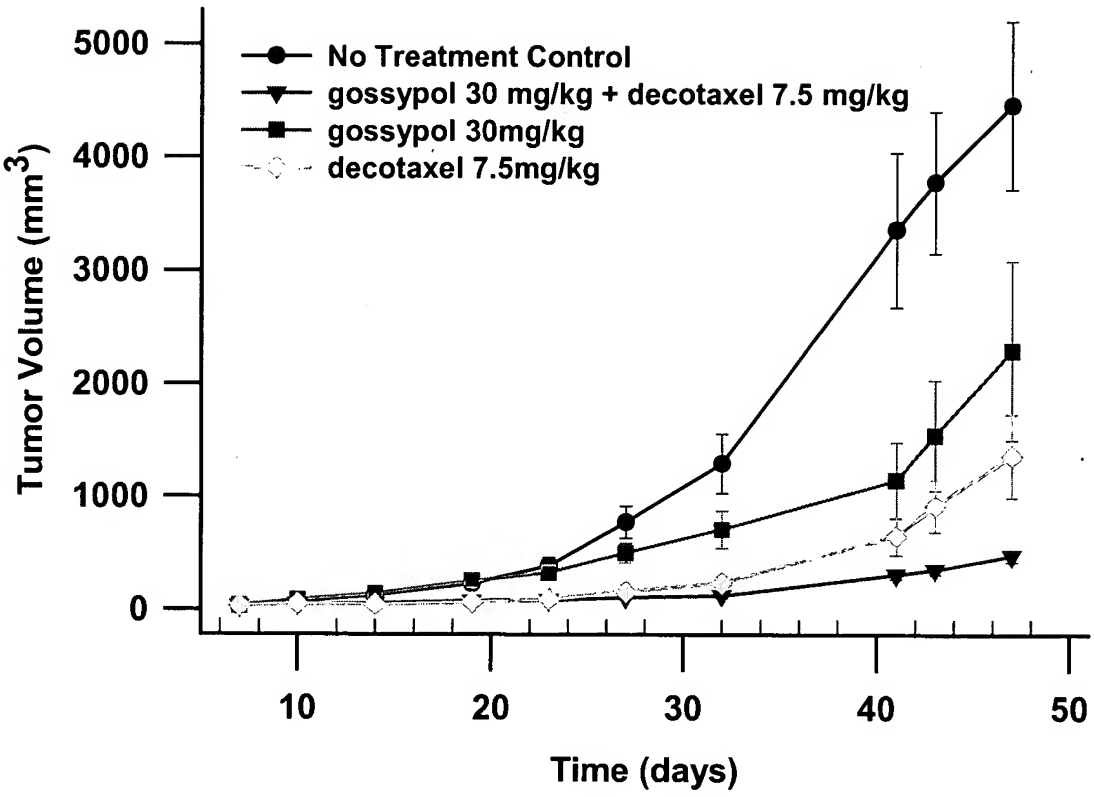
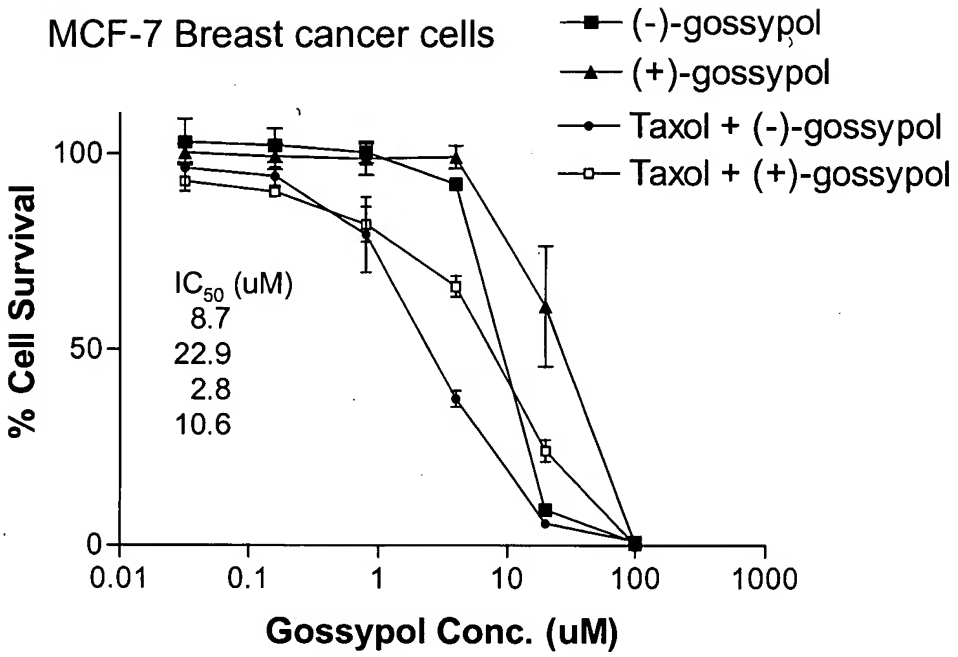

No Treatment Control
gossypol 30 mg/kg + decotaxel 7.5 mg/kg
gossypol 30mg/kg
decotaxel 7.5mg/kg
Tumor Volume (mm3)
5000
4000
3000
2000
1000
0
10
20
30
40
50
Time (days)

Figure 16



This experiment used 100:1 ratio between (-)-gossypol and Taxol, and between (+)-gossypol and Taxol

Figure 17A

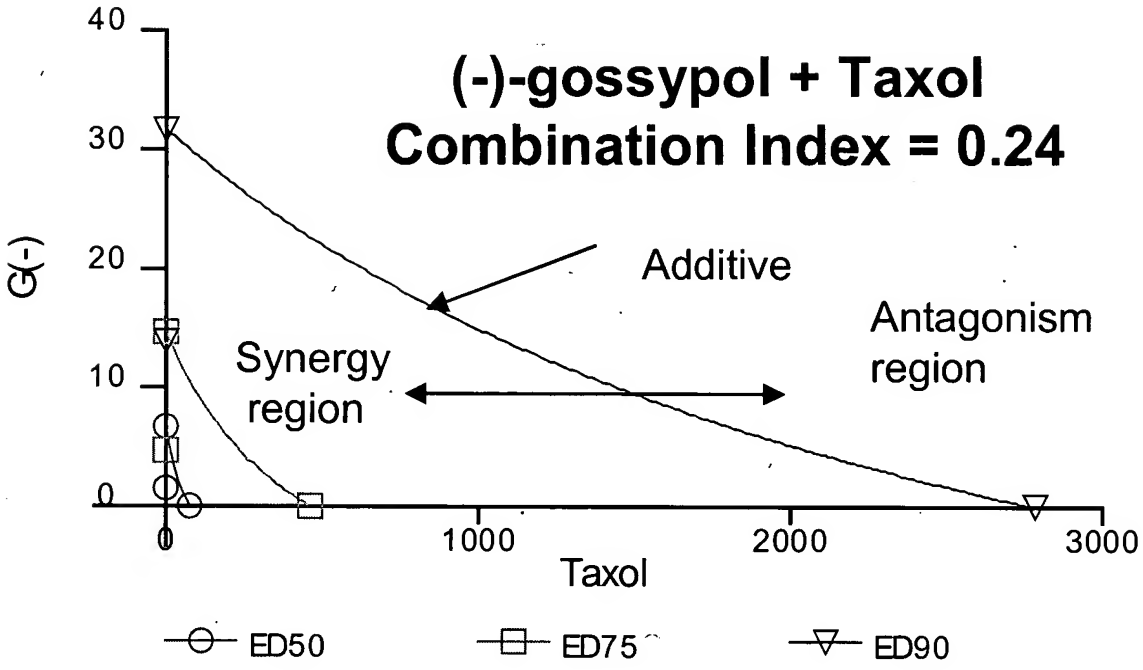

(-)-gossypol + Taxol
Combination Index = 0.24
40
30
20
10
0
G(-)
Additive
Antagonism region
Synergy region
0
1000
2000
3000
Taxol
ED50
ED75
ED90

Figure 17B

MDA-MB-231 DOX + G- 1:2.5uM

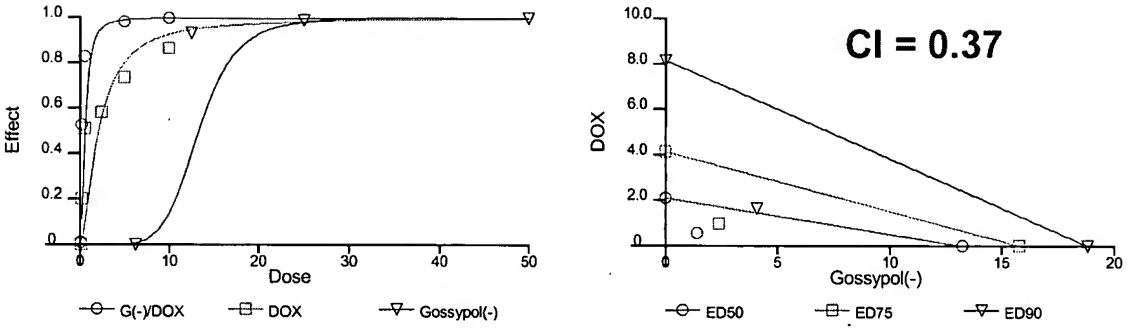


Figure 18

Effect of (-)-gossypol on inhibition of tumor growth of human breast cancer xenograft MDA-231

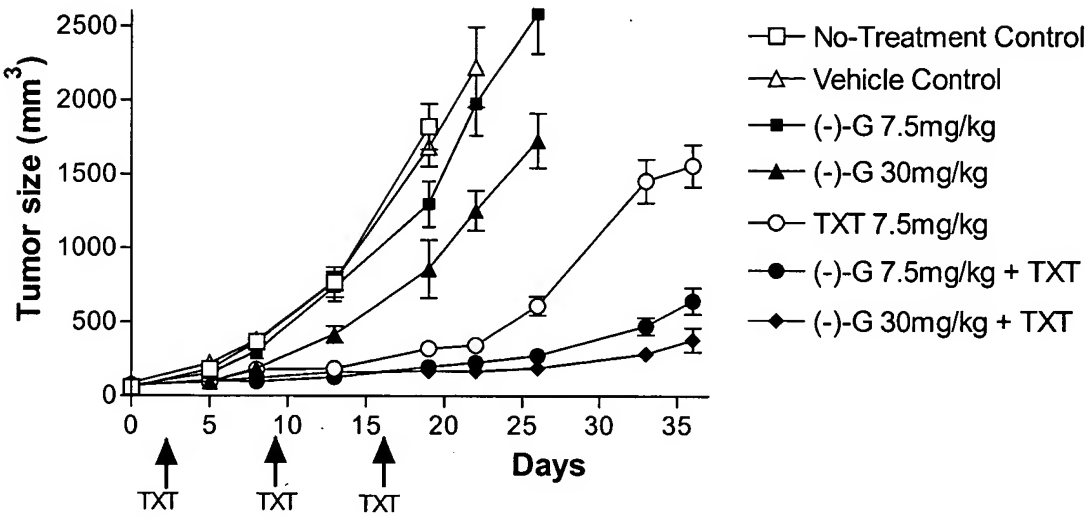


Figure 19

Effect of (-)-gossypol on inhibition of tumor growth of human breast cancer xenograft MDA-231

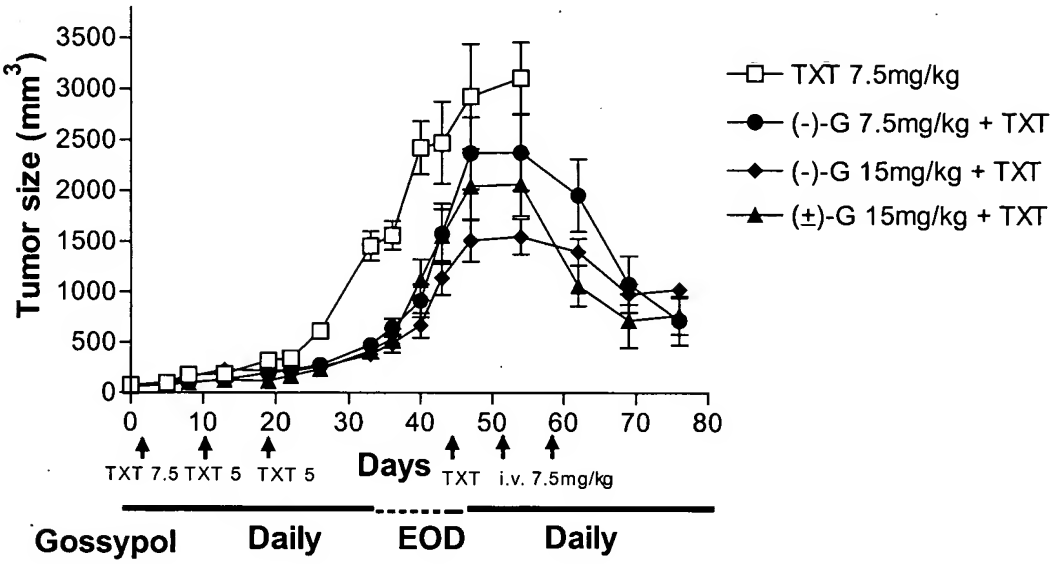


Figure 20

Effect of (-)-gossypol on inhibition of tumor growth of human non-samll cell lung carcinoma cell xenograft A-549

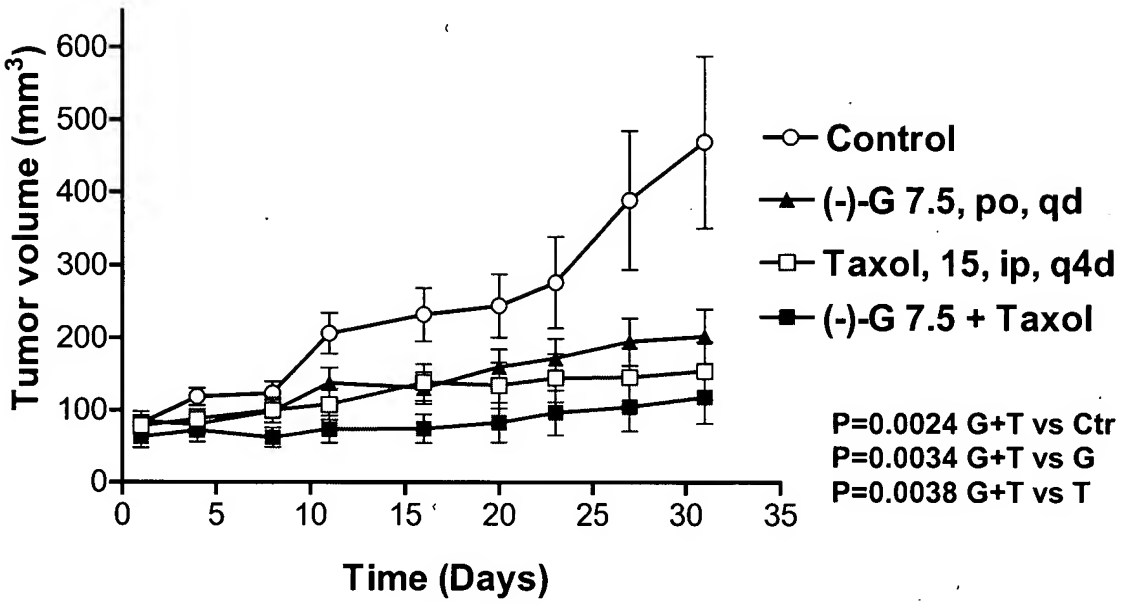


Figure 21

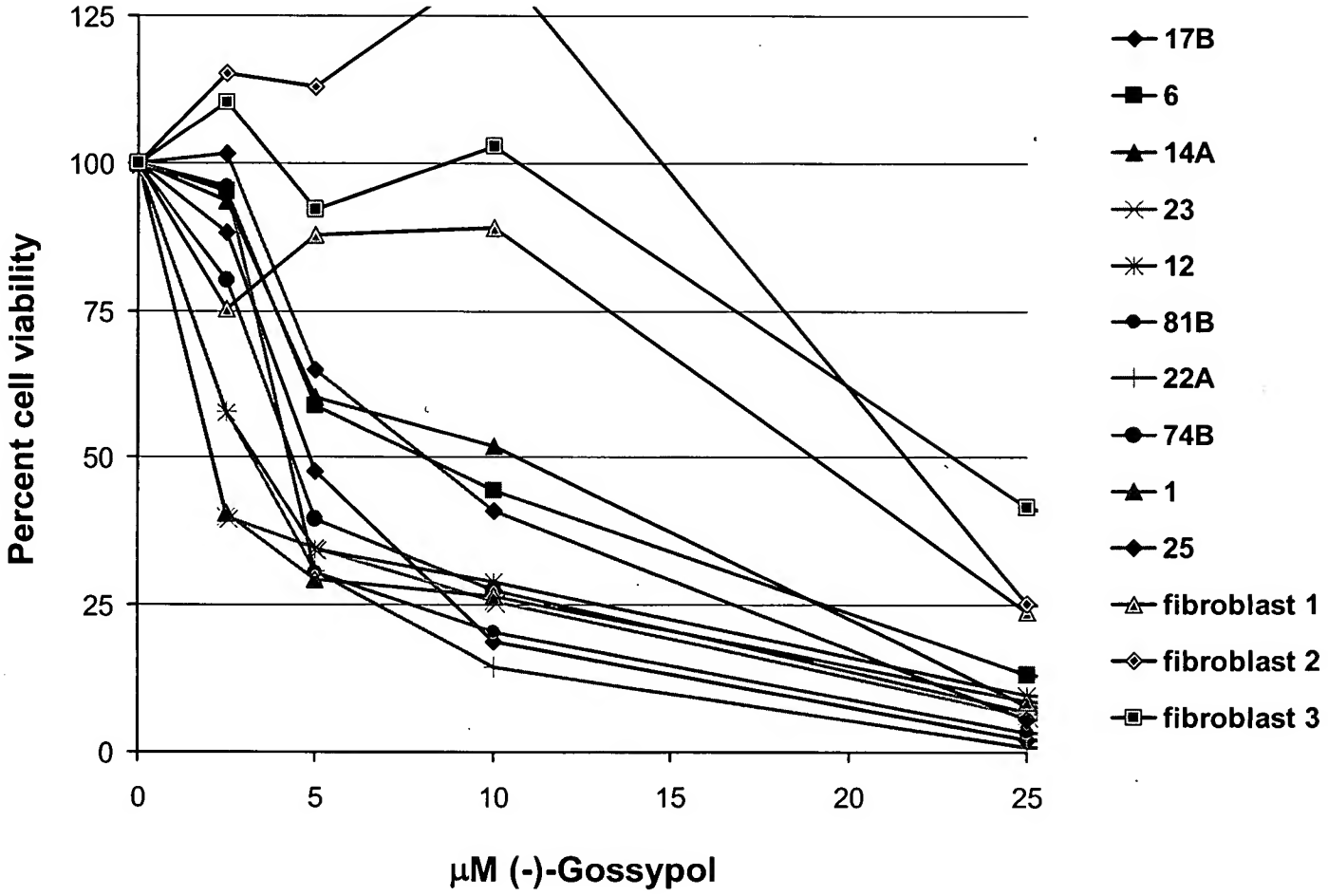

125
100
75
50
25
0
Percent cell viability
0
5
10
15
20
25
µM (-)-Gossypol
17B
6
14A
23
12
81B
22A
74B
1
25
fibroblast 1
fibroblast 2
fibroblast 3

Figure 22

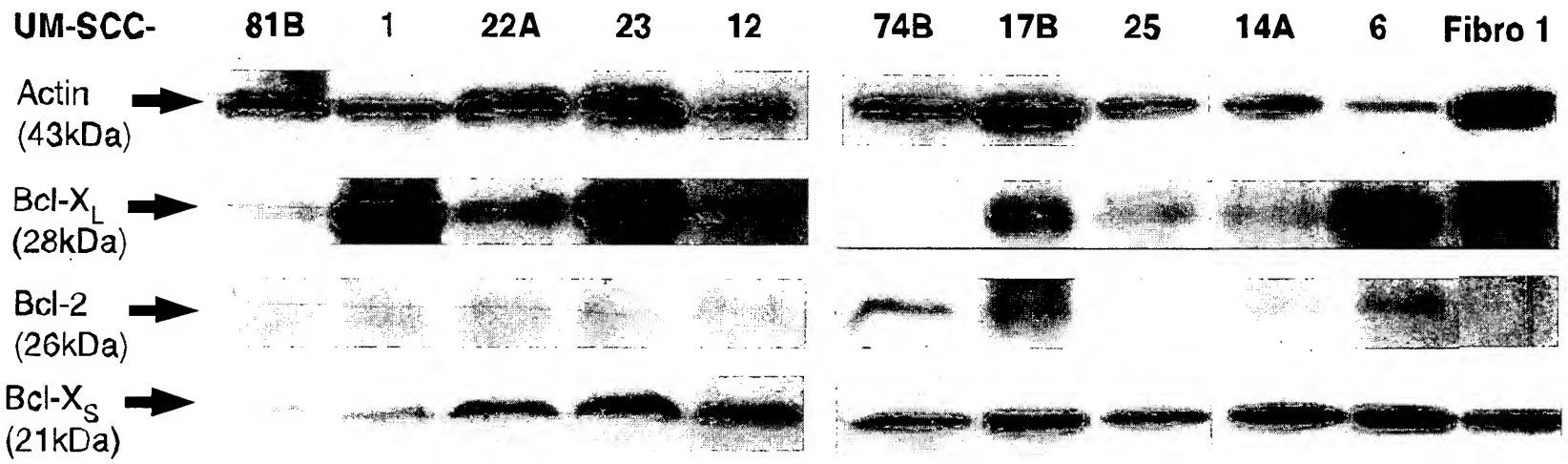

UM-SCC-
81B
1
22A
23
12
74B
17B
25
14A
6
Fibro 1
Actin
(43kDa)
Bcl-X$_L$
(28kDa)
Bcl-2
(26kDa)
Bcl-X$_S$
(21kDa)

Figure 23

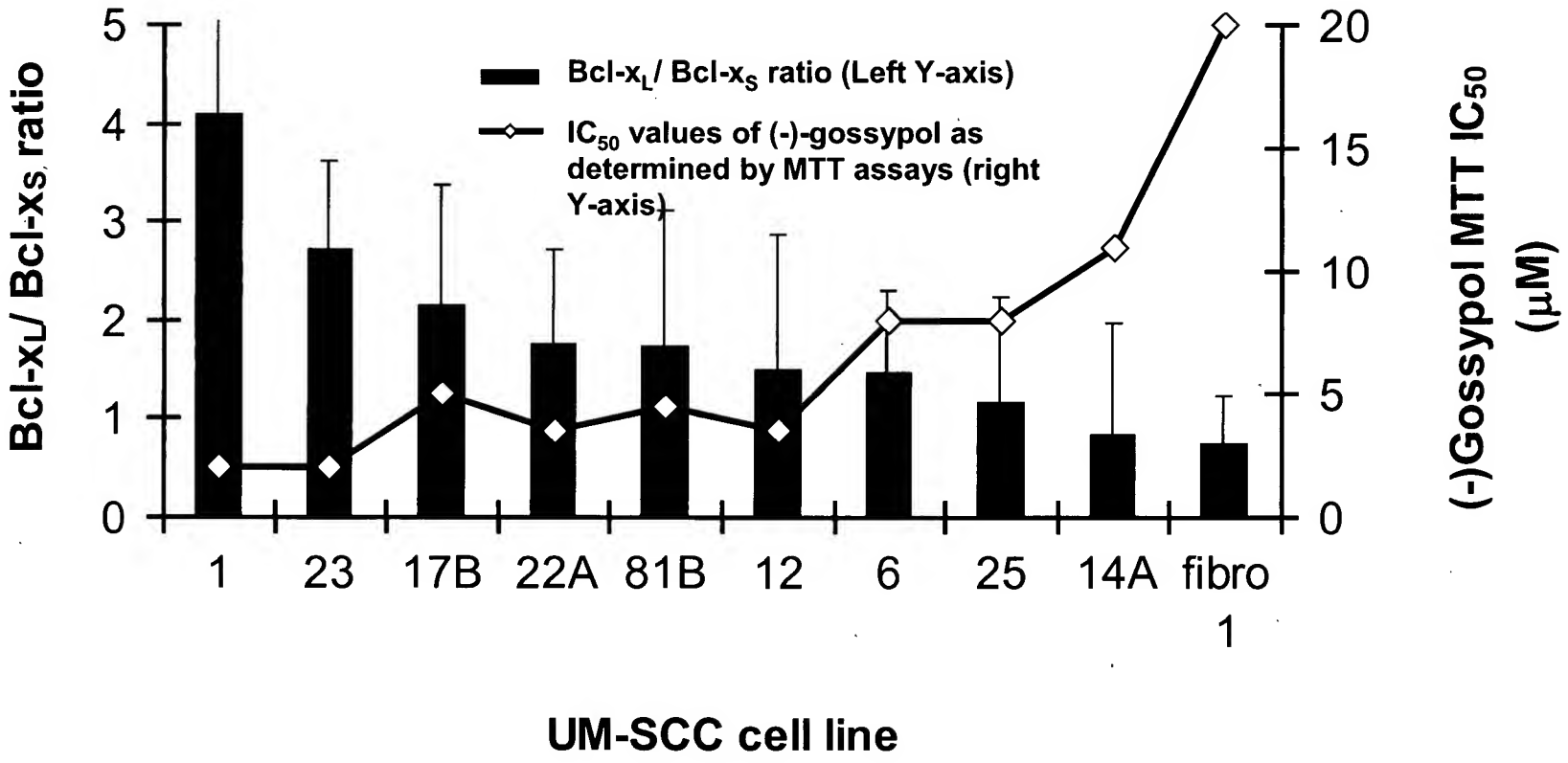

Bcl-xL/ Bcl-xS ratio
5
4
3
2
1
0
Bcl-xL/ Bcl-xS ratio (Left Y-axis)
IC50 values of (-)-gossypol as determined by MTT assays (right Y-axis)
20
15
10
5
0
(-)Gossypol MTT IC50 (μM)
1
23
17B
22A
81B
12
6
25
14A
fibro 1
UM-SCC cell line

Figure 24A

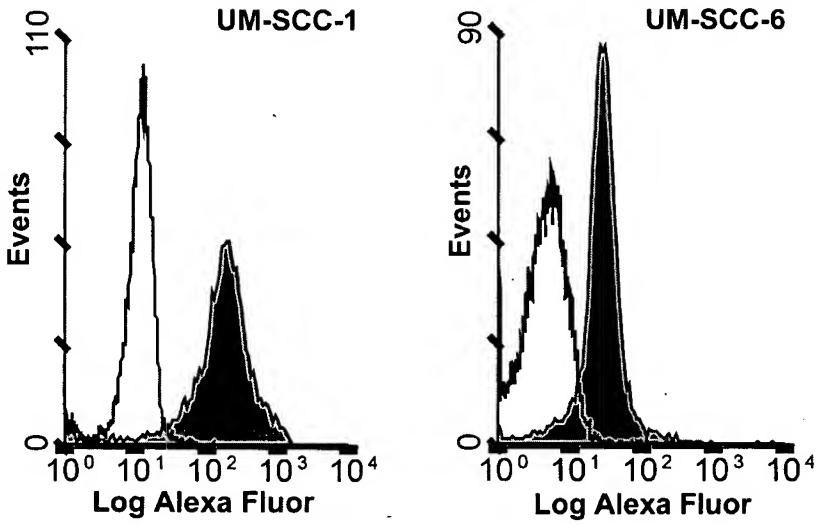

UM-SCC-1
110
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
UM-SCC-6
90
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

Figure 24B

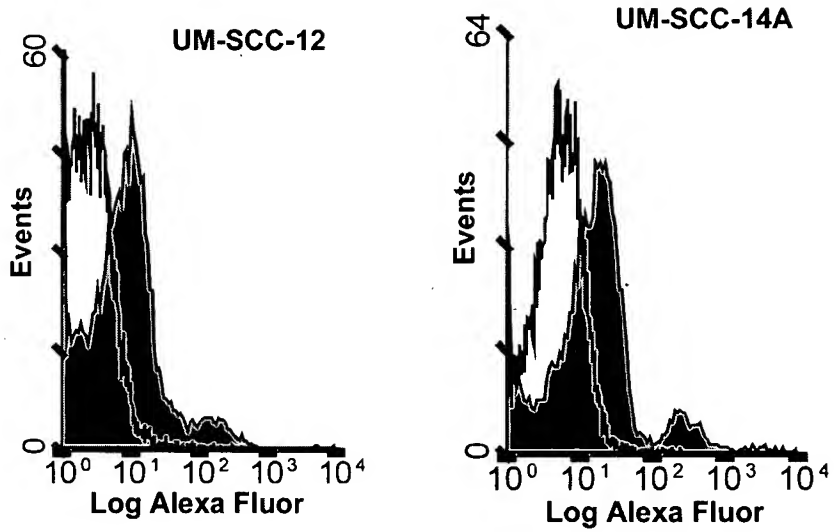

UM-SCC-12
60
0
Events
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
UM-SCC-14A
64
0
Events
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

Figure 24C

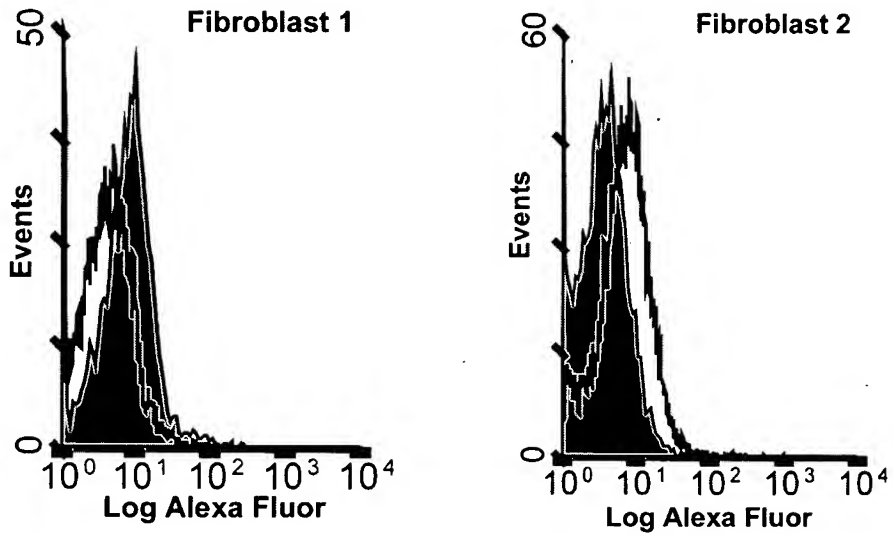
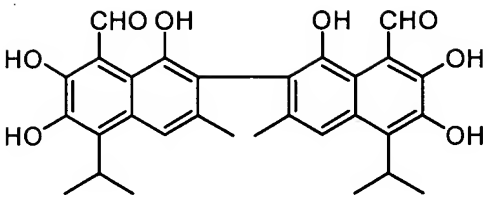
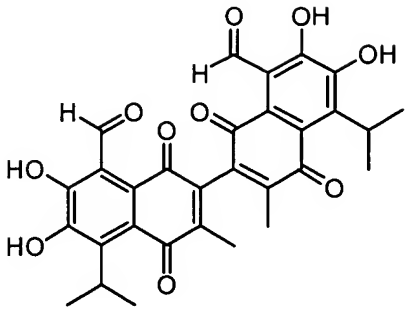

Fibroblast 1
50
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor
Fibroblast 2
60
Events
0
10^0 10^1 10^2 10^3 10^4
Log Alexa Fluor

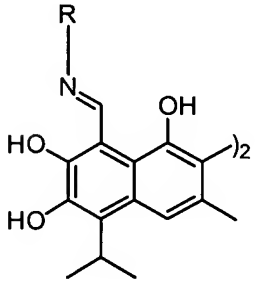
Figure 25



Gossypol

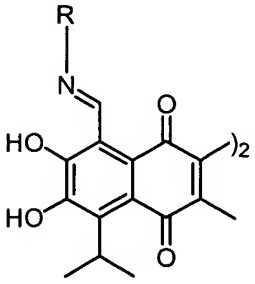


Gossypolone



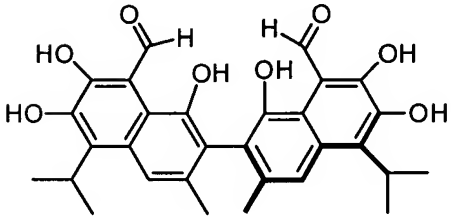
R = aliphatic or aromatic group

Schiff's base of Gossypol

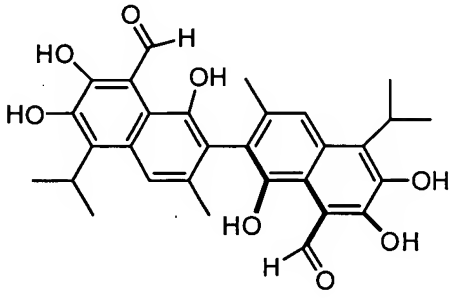


R = aliphatic or aromatic group

Schiff's base of Gossypolone



(-)-(*R*)-Gossypol



(+)-(*S*)-Gossypol

Figure 26

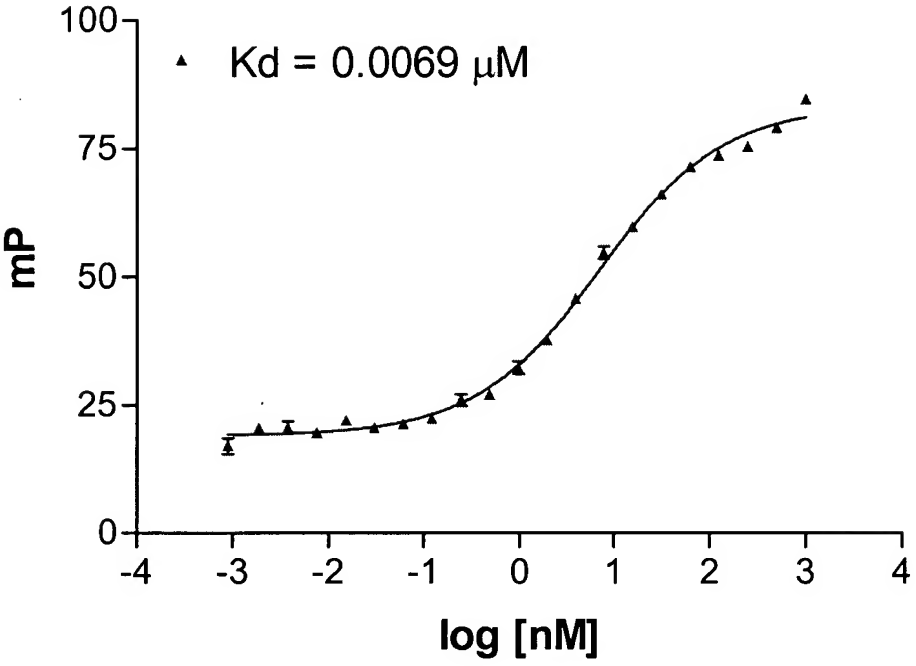


Figure 27

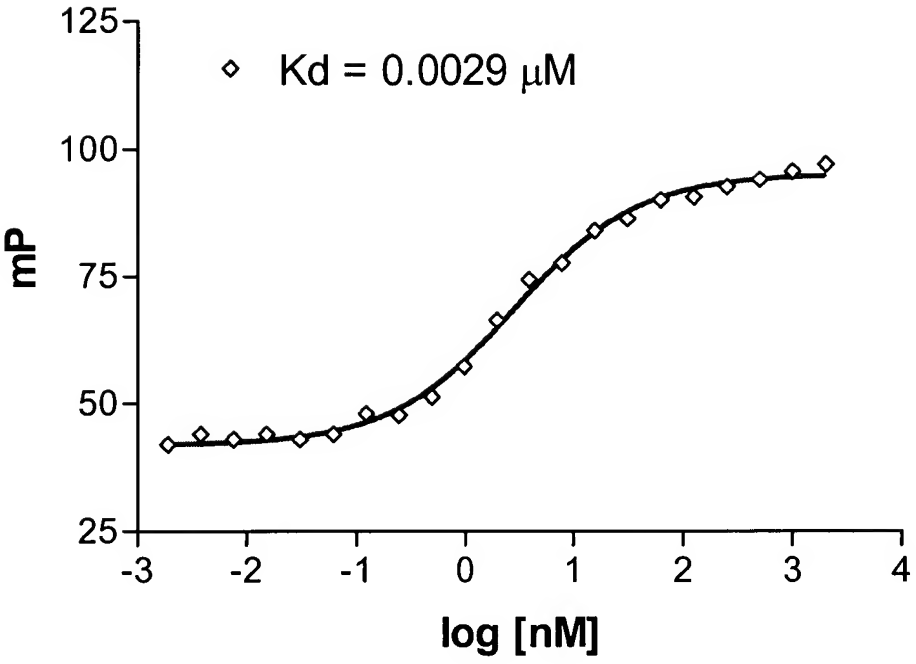


Figure 28A

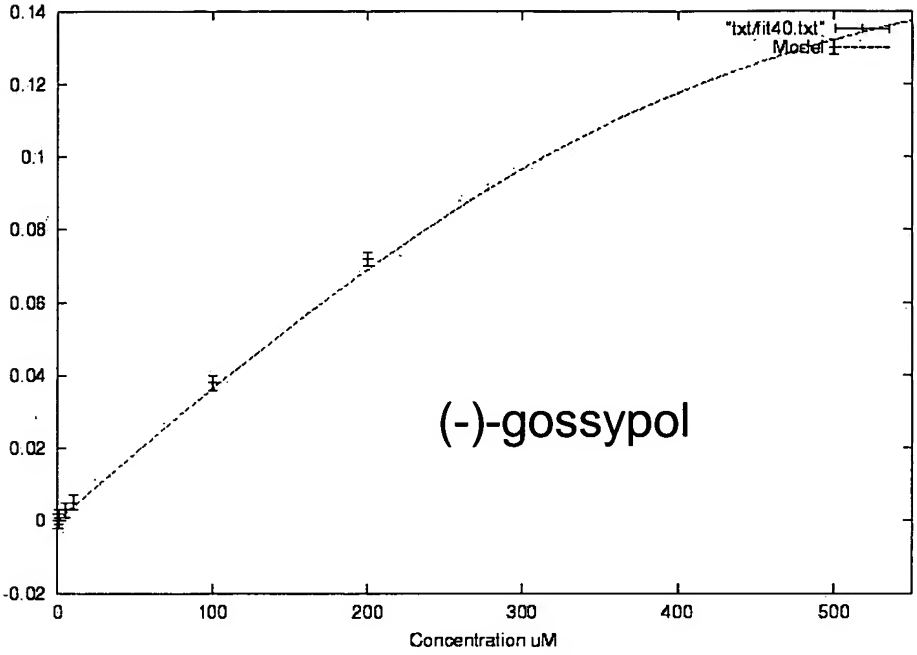

0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
100
200
300
400
500
Concentration uM
"txt/fit40.txt"
Model
(-)-gossypol

Figure 28B

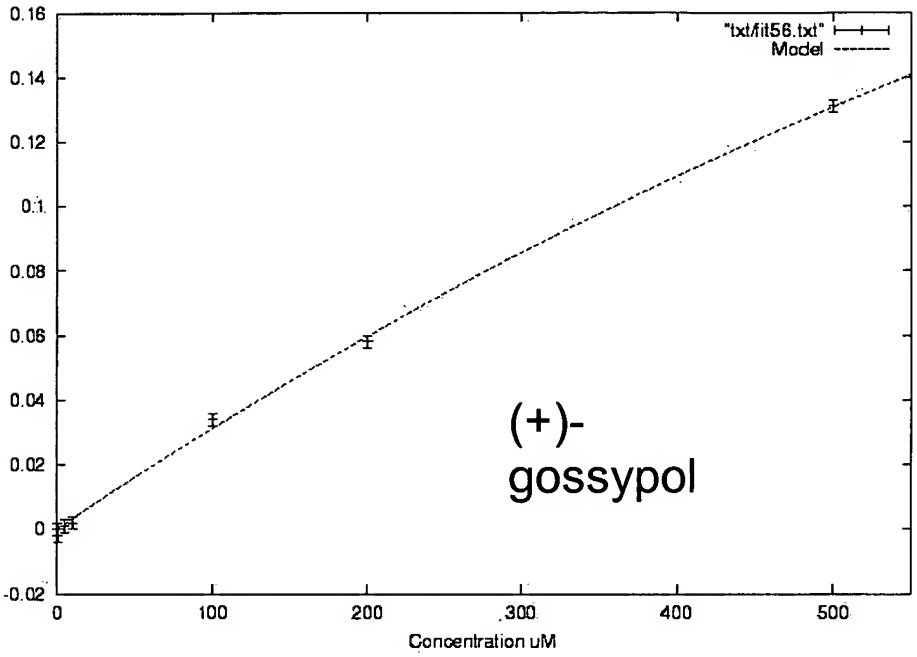

"txt/fit56.txt"
Model
(+)-
gossypol
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
-0.02
0
100
200
300
400
500
Concentration uM

Figure 29

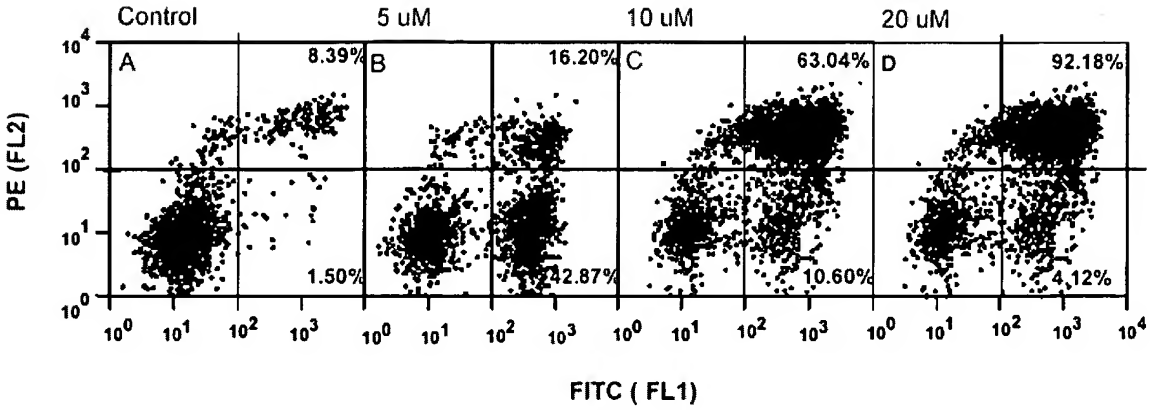

Control
5 uM
10 uM
20 uM
A
8.39%
1.50%
B
16.20%
2.87%
C
63.04%
10.60%
D
92.18%
4.12%
PE (FL2)
FITC (FL1)

Figure 30

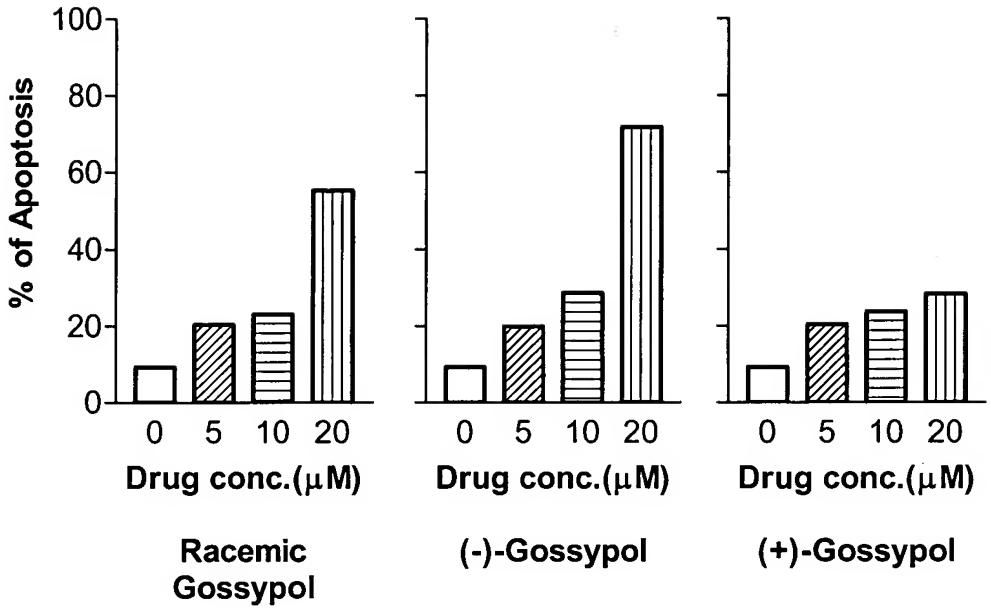

% of Apoptosis
100
80
60
40
20
0
0 5 10 20
Drug conc.(μM)
Racemic Gossypol
0 5 10 20
Drug conc.(μM)
(-)-Gossypol
0 5 10 20
Drug conc.(μM)
(+)-Gossypol

Figure 31

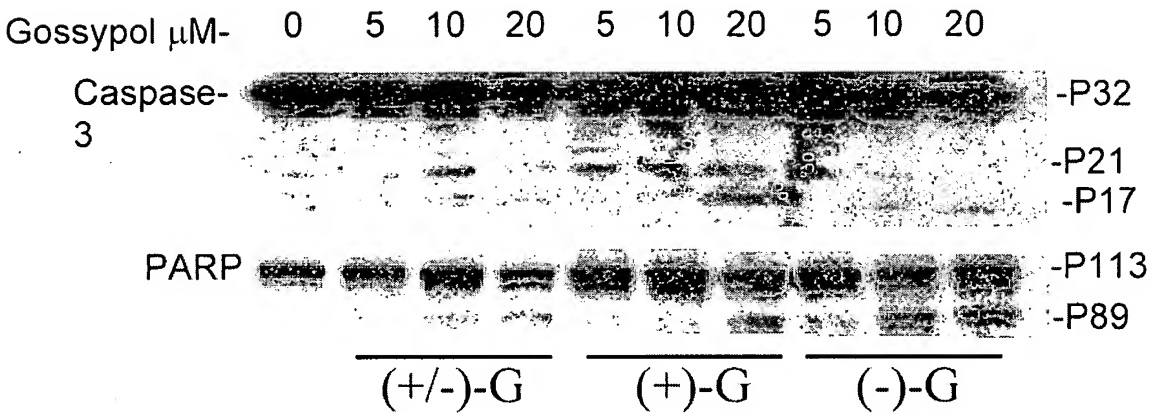

Gossypol μM- 0 5 10 20 5 10 20 5 10 20
Caspase-3
-P32
-P21
-P17
PARP
-P113
-P89
(+/-)-G
(+)-G
(-)-G

Figure 32A

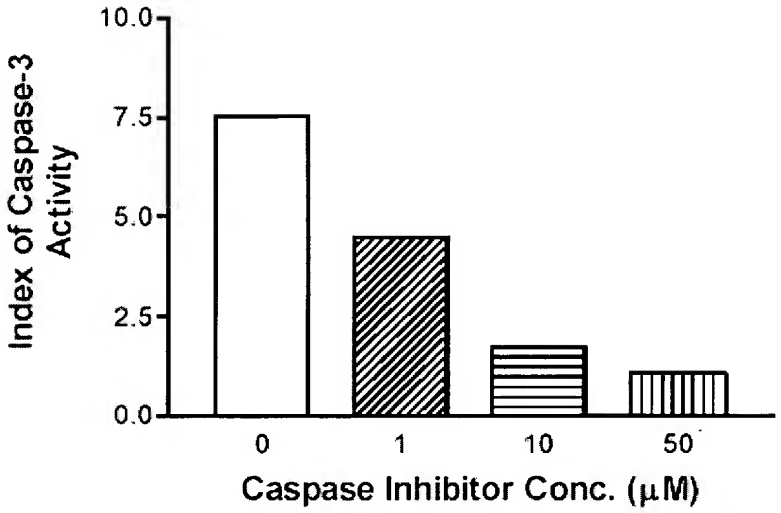


Figure 32B

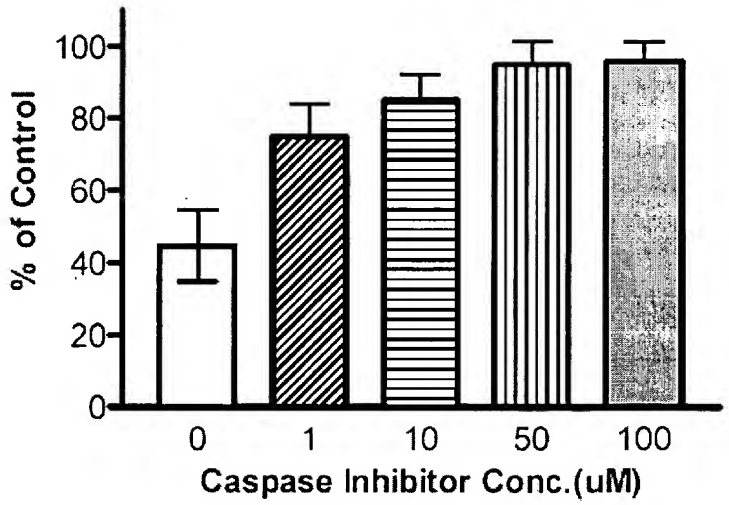


Figure 33

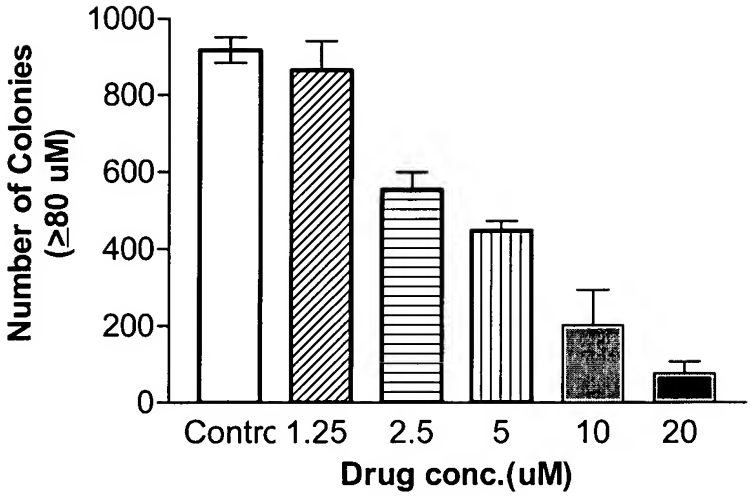

Number of Colonies (≥80 uM)
1000
800
600
400
200
0
Contrc
1.25
2.5
5
10
20
Drug conc.(uM)

Figure 34

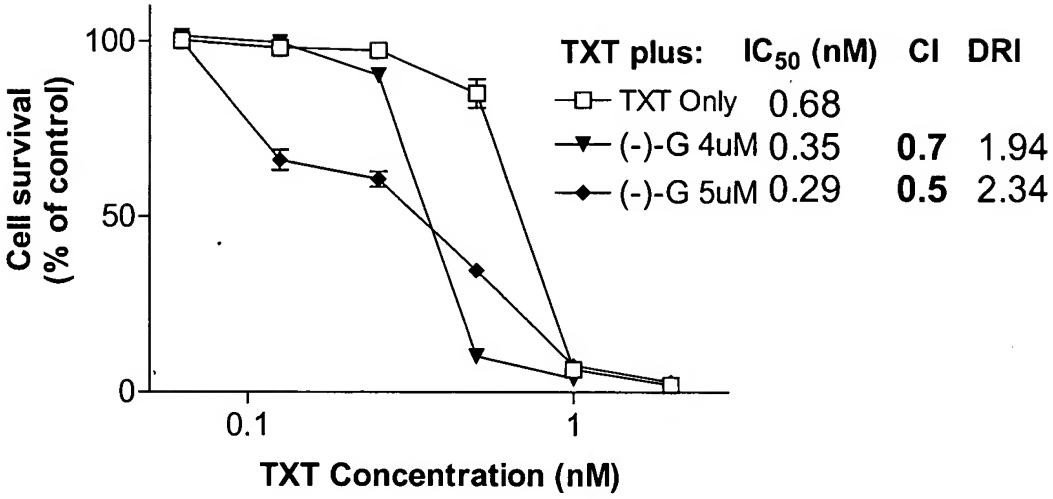

100
50
0
0.1
1
Cell survival (% of control)
TXT Concentration (nM)
TXT plus: IC50 (nM) CI DRI
TXT Only 0.68
(-)-G 4uM 0.35 0.7 1.94
(-)-G 5uM 0.29 0.5 2.34

Figure 35A

In vitro effects of gossypol-(-) in combination with various doses of radiation on PC-3 clonogenic assays

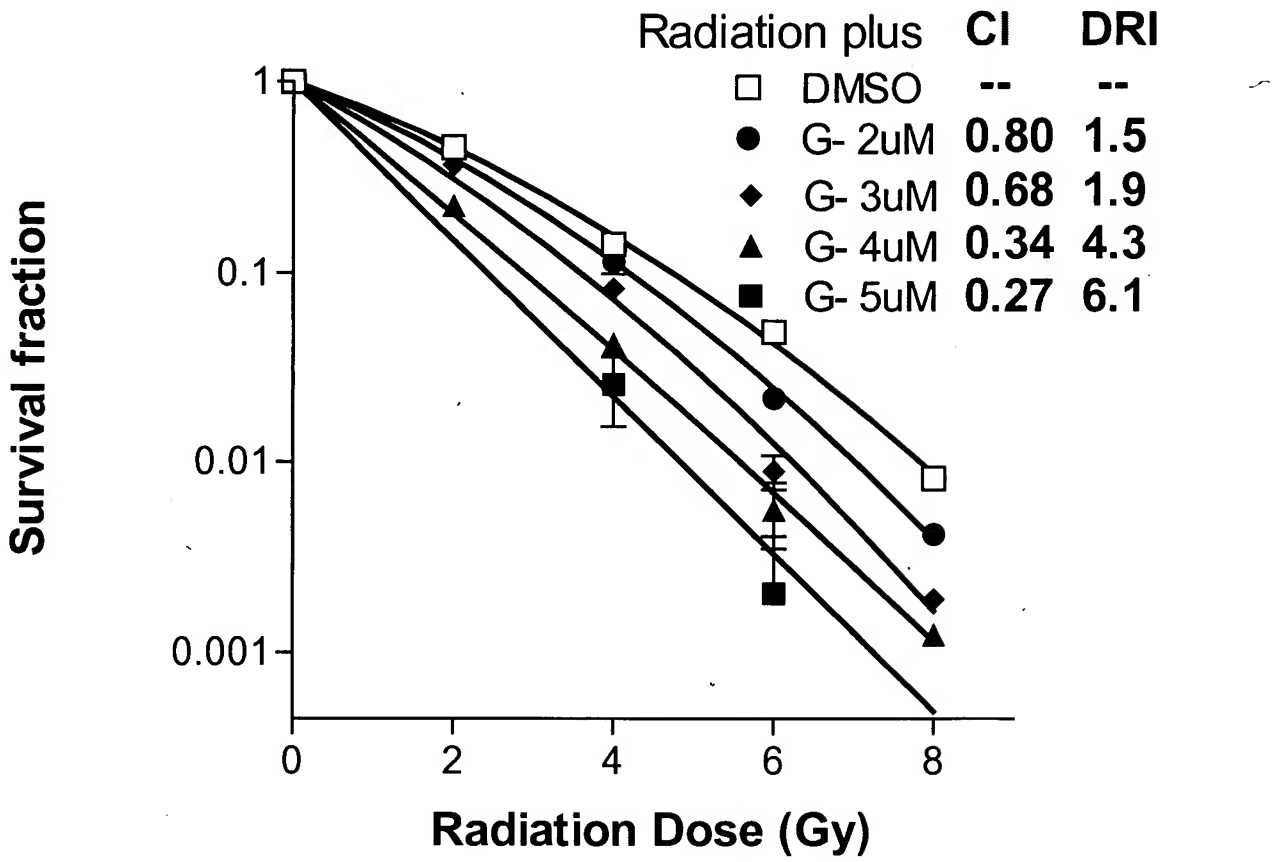


Figure 35B

G- μM	0	1	2	3	4	5
D bar = Mean inactivation dose	**2.22**	**2.06**	**1.95**	**1.63**	**1.26**	**1.05**
Gy(1%)= Dose required for 1% cell survival	**7.84**	**7.11**	**7.03**	**6.25**	**5.59**	**4.84**
SF(2Gy)= Survival fraction at 2Gy	**0.45**	**0.43**	**0.4**	**0.31**	**0.21**	**0.15**

Figure 36

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

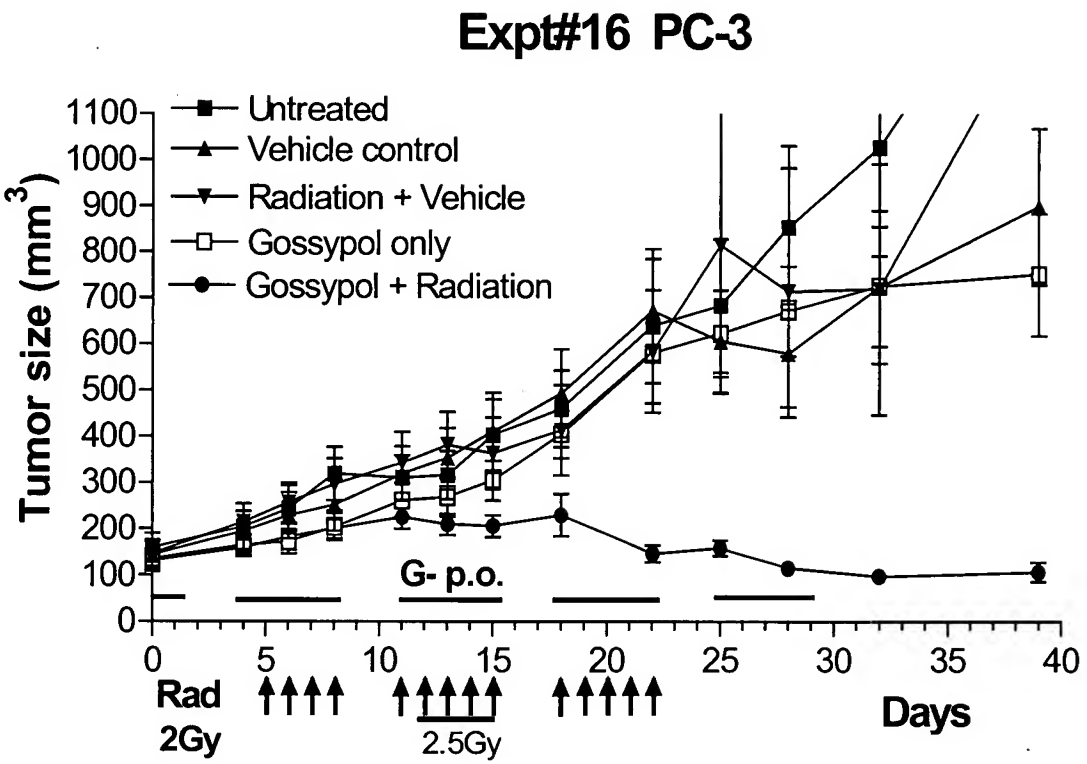


Figure 37

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

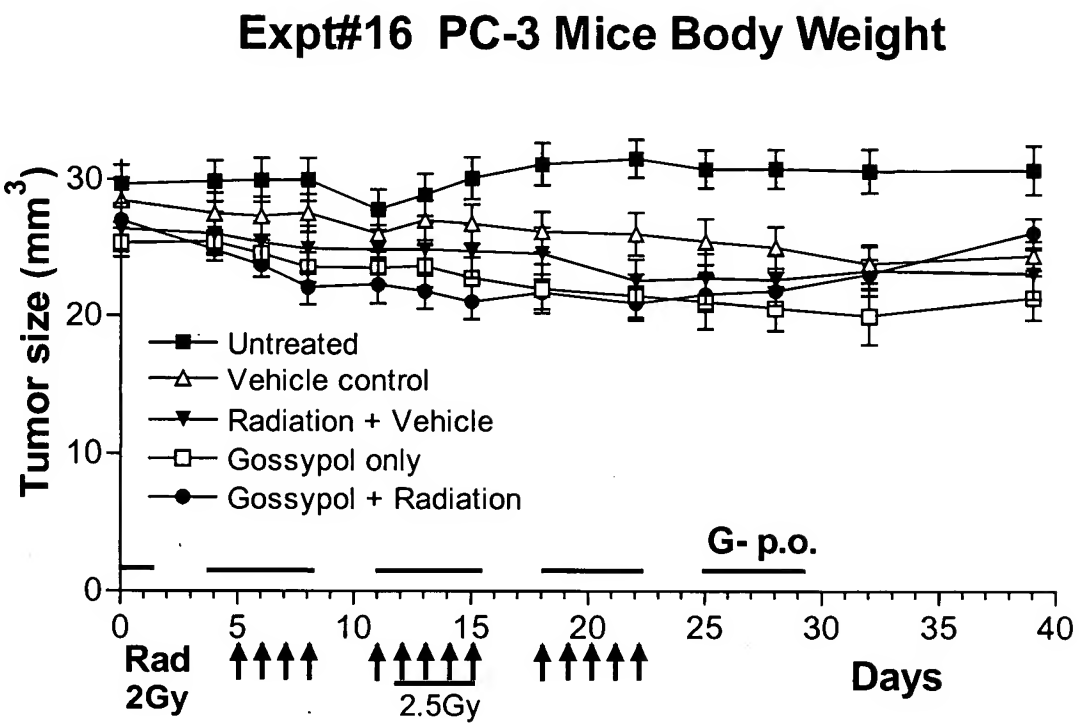


Figure 38

(-)-gossypol in combination with radiation in an androgen-independent prostate PC-3 xenograft model

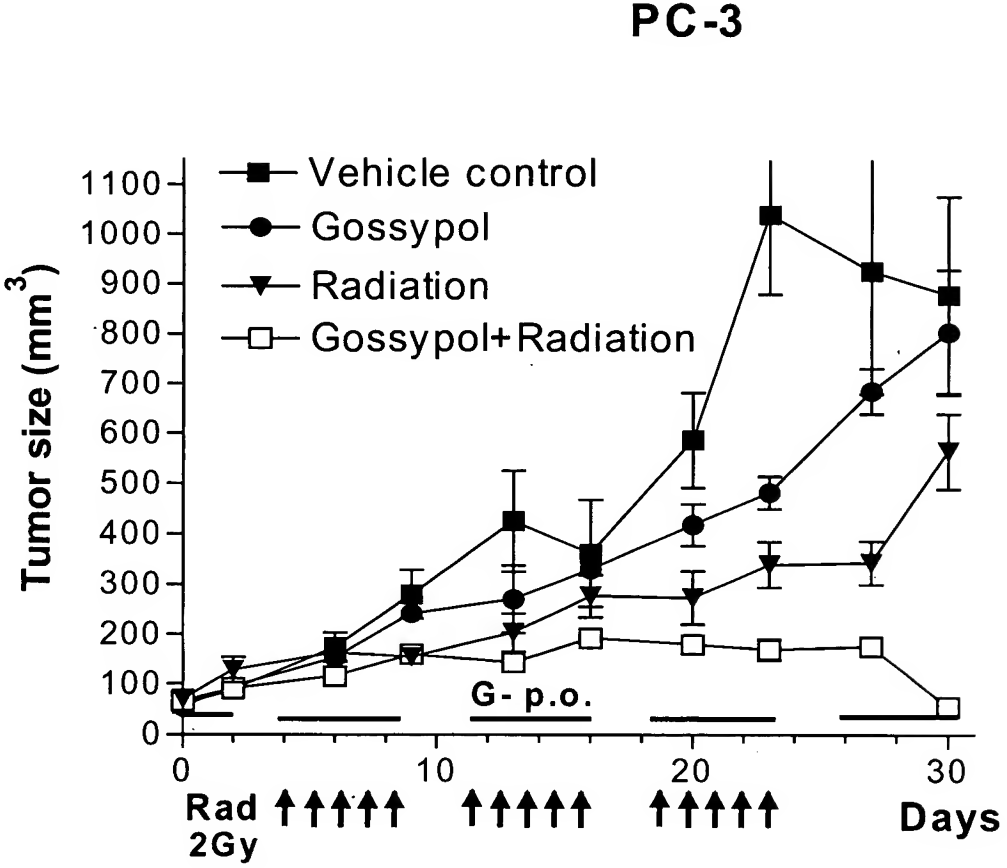
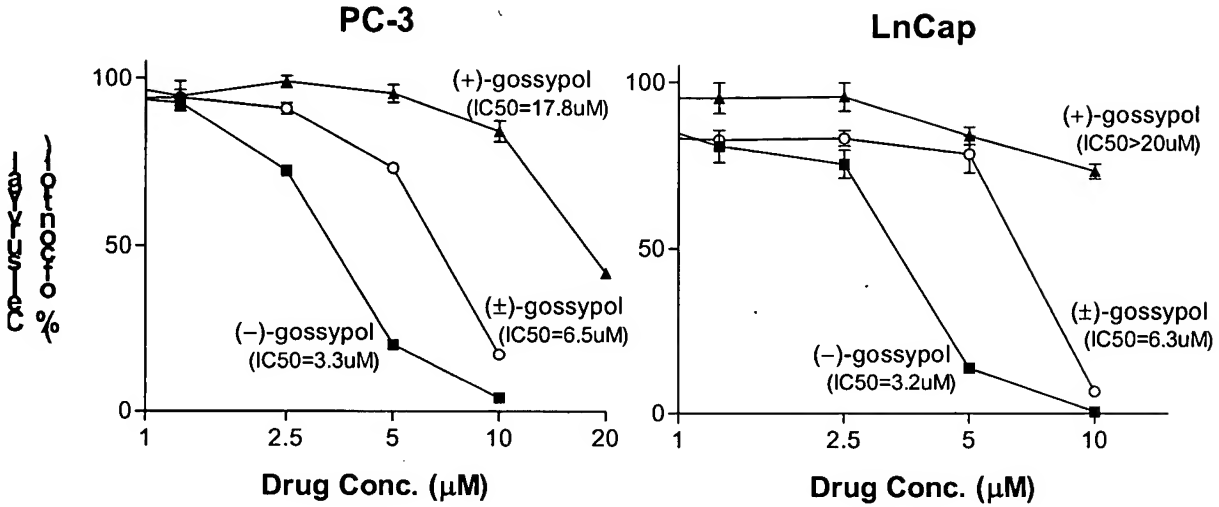
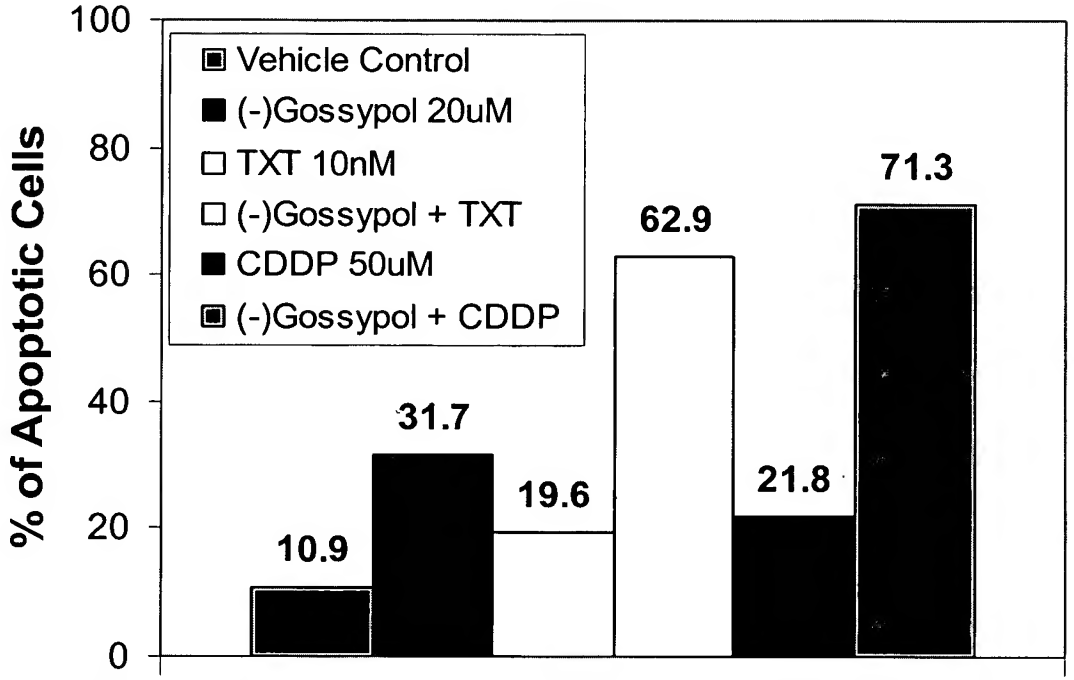


Figure 39



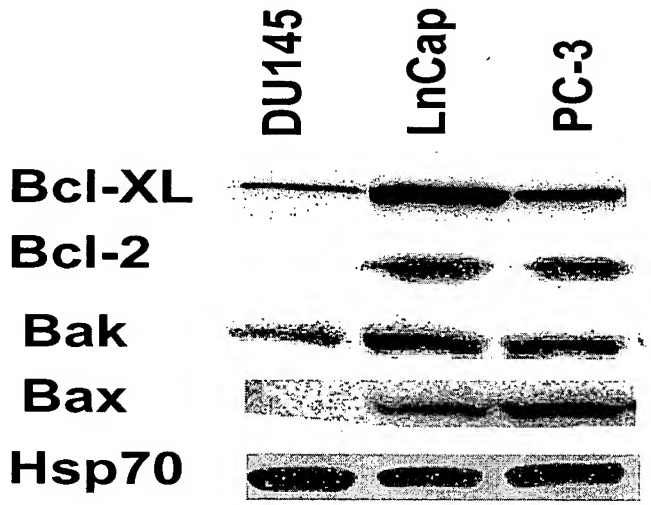
Prostate cancer cell growth inhibition by gossypol. PC-3 and LnCap cells in 96-well plates were treated in triplicates with gossypol and its enantiomers. MTT-based 5-day cell proliferation assay was performed and IC50, drug concentration that inhibited 50% of cell growth, was calculated. (-)-gossypol is 5-10 times more potent that (±)-gossypol, 2 times more potent than (±)-gossypol, in both cell lines.

Figure 40



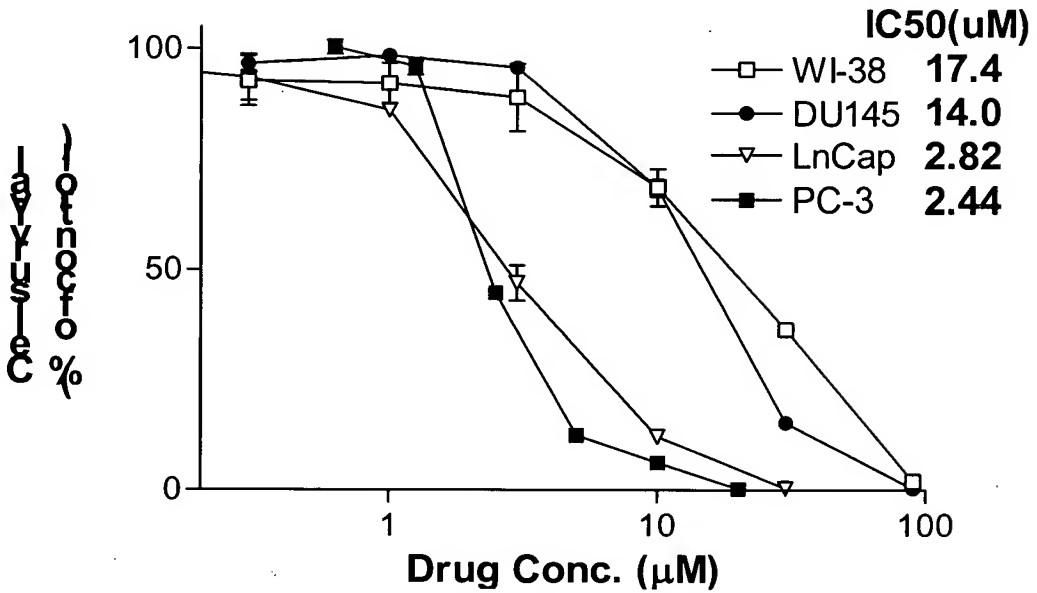
(-)-Gossypol enhances chemotherapy-induced apoptosis in human prostate cancer PC-3 cells. Cells were treated with (-)-gossypol alone or in combination with TXT or CDDP for 48hr, then stained with Annexin V-FITC and PI for flow cytometry. Values are % of apoptotic cells.

Figure 41



Basal levels of Bcl-2 family proteins expression in three prostate cancer cell lines. HSP70: heat shock protein 70kDa for gel loading control.

Figure 42



Cytotoxicity of (-)-gossypol on prostate cancer cells. MTT-based 5-day cell proliferation assay was performed and IC50, drug concentration that inhibited 50% of cell growth, was calculated.

Figure 43A

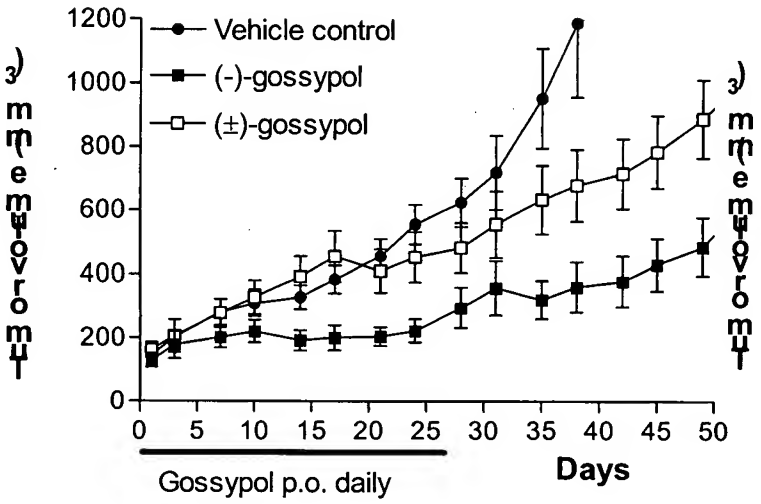
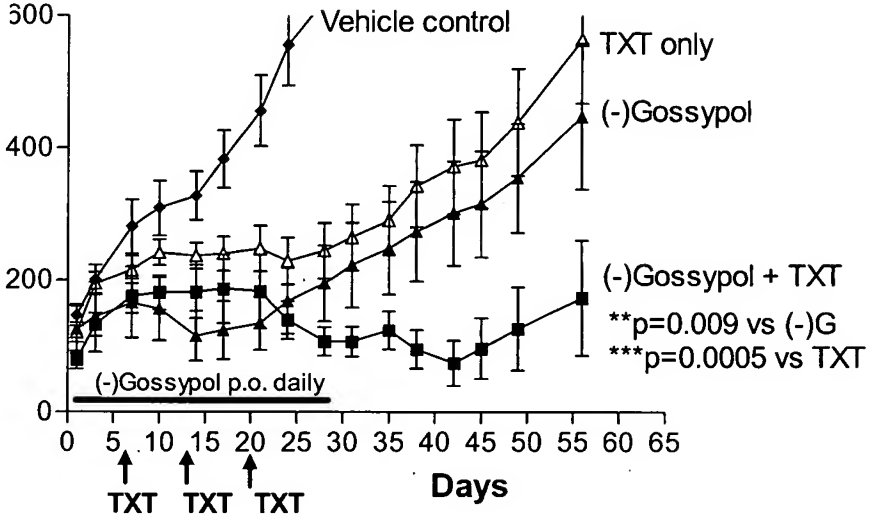


Figure 43B



In vivo anti-tumor activity of gossypol in human prostate cancer PC-3 xenograft model. **A:** 15mg/kg (±)- or (-)-gossypol p.o. daily for 26 days. (-)-gossypol is more potent than (±)-gossypol ($P<0.001$). **B:** Tumor growth inhibition by (-)-gossypol was significantly enhanced when used in combination with docetaxel (TXT). **Student's t-test.

Figure 44

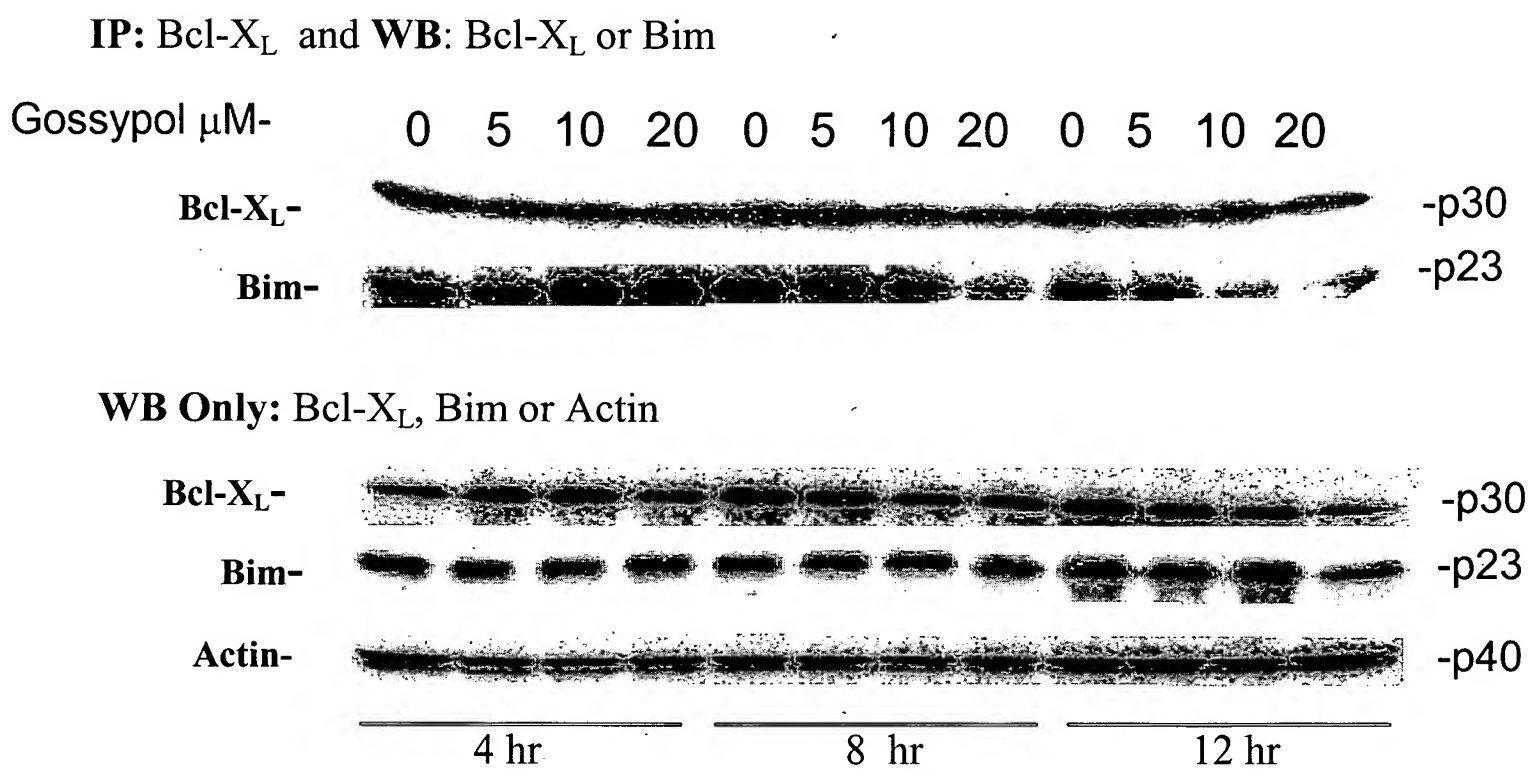

IP: Bcl-X$_L$ and WB: Bcl-X$_L$ or Bim
Gossypol μM-
0 5 10 20 0 5 10 20 0 5 10 20
Bcl-X$_L$-
-p30
Bim-
-p23
WB Only: Bcl-X$_L$, Bim or Actin
Bcl-X$_L$-
-p30
Bim-
-p23
Actin-
-p40
4 hr
8 hr
12 hr

Figure 45

Competitive binding curve of apogossypol against Bcl-2

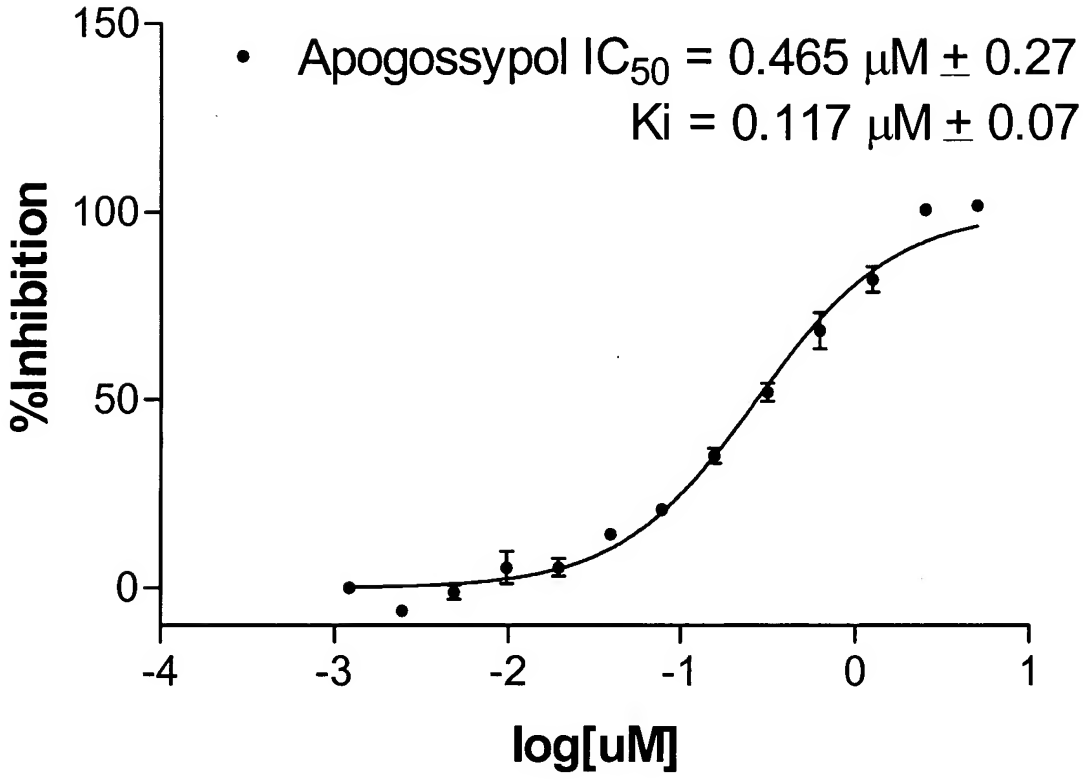


Figure 46

Competitive binding curve of apogossypol against Bcl-X_L.